



INSTITUTE FOR DEFENSE ANALYSES

**Research and Development Strategies  
for the Current and Future Medical  
Treatment of Radiation Casualties**

Katherine M. Sixt  
Forrest R. Smith, Jr.  
Deborah Kim  
Carl A. Curling, Project Leader

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## **Executive Summary**

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In support of the United States (U.S.) Army Office of the Surgeon General (OTSG) and the Joint Requirements Office for Chemical, Biological, Radiological, and Nuclear (CBRN) Defense (J-8/JRO), the Institute for Defense Analyses (IDA) was asked to “review the potential for the use of radioprotectant drugs and radiation injury treatments to change the casualty estimate and require revisions to existing policy, doctrine, and technical documentation.” In this paper, IDA examines the current medical treatments for acute radiation syndrome (ARS), and it explores the future drug development and research and development (R&D) strategies that the Department of Defense (DOD) can use to assist in the discovery and fielding of new medical countermeasures for ARS. Examples of potential countermeasures employing some of these development strategies are also described.

The traditional drug development model has been the primary focus of prior DOD investment for bringing an ARS countermeasure to approval by the Food and Drug Administration (FDA). However, this model is costly, time-consuming, and risky, as very few drugs that enter Phase 1 clinical trials ever attain approval. Alternate drug development strategies, such as repurposing drugs approved for other indications or combining drugs for codevelopment in the treatment of ARS are explored in this paper.

Current treatment of supportive care and leukocyte growth factors mitigate the hematopoietic (HP) ARS that occurs at lower levels of radiation exposure. Therefore, focus should be shifted to development of countermeasures to treat gastrointestinal (GI) ARS that results from higher doses of radiation. In addition to the alternate drug development strategies described earlier, research strategies, such as supporting development of drug therapies for treatment of GI disorders with similar mechanisms to GI ARS and developing high-throughput screening assays for GI ARS, are discussed in this paper.

Since the ultimate goal of fielding a medical countermeasure for ARS would be to reduce fatalities in a radiation incident, two illustrative examples of a nuclear detonation in either a civilian center—Washington, DC—or a military scenario—the Heavy Brigade Combat Team (HBCT)—are described. Fatalities are estimated using the methodology of the *Allied Medical Publication 8(C) [AMedP-8(C)]: NATO Planning Guide for the Estimation of CBRN Casualties*. The effect of either supportive care or supportive care and filgrastim on the number of fatalities in each scenario is estimated. This model can be

used to estimate the effects of other countermeasures if a dose reduction factor (DRF) has been calculated at the median lethal dose ( $LD_{50}$ ) for the countermeasure.

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## **1. Introduction**

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Radiation medical countermeasures, such as radioprotectant drugs and radiation injury treatments, have the potential to mitigate the effects of a radiation or nuclear incident on the health and survival of those exposed to ionizing radiation (IR). However, to date no medical countermeasures have been approved for use in the treatment of acute radiation syndrome (ARS), which is the disease state that results from body exposure to high doses of IR.

Hundreds, or even thousands, of compounds have shown some ability to improve the health and survival of humans, animals, or cells following IR exposure. Likely many other prospective ARS treatments remain undiscovered. To determine which potential therapies could have the most significant effect on the health of humans requires an approach that not only focuses on those that are most efficacious, but it also should consider their safety profiles, methods of administration, storage requirements, and congruencies with the United States (U.S.) regulatory, civilian, and military policies. Furthermore, the research and development (R&D) pathways toward therapy identification and subsequent approval should also align with these policies, while accounting for cost, time to approval, and risk or likelihood of approval. This paper attempts to address these considerations to assist the sponsor of this work, the U.S. Army Office of the Surgeon General (OTSG), and the Department of Defense (DOD) more broadly, in prioritizing the research efforts required to obtain an effective and safe medical countermeasure against the effects of IR.

Radiation medical countermeasures may reduce the overall number of individuals who die or become severely injured as a consequence of a radiation or nuclear incident. The analysis presented in this paper was completed by the Institute for Defense Analyses (IDA) under Task Order CA-6-3079 “CBRN Casualty Estimation and Support to the Medical CBRN Defense Planning & Response Project,” Subtask 2 “Update Agents/Materials into *AMedP-8(C)* Casualty Estimation Methodology.” The task order requires IDA to “review the potential for the use of radioprotectant drugs and radiation injury treatments to change the casualty estimate and require revisions to existing policy, doctrine, and technical documentation.” Since no radioprotectants or radiation injury treatments are currently approved by the U.S. Food and Drug Administration (FDA) for the treatment of ARS, this paper describes the current ARS treatment of supportive care

and the off-label use of filgrastim.<sup>1</sup> Additionally, this paper outlines strategies beyond the traditional R&D pharmaceutical model that DOD could implement with less cost, less time to approval, and less risk for acquiring future treatments for ARS, and it describes some potential therapies that are still being tested for safety and efficacy. Lastly, an illustrative example of how supportive care alone, or in conjunction with filgrastim, could affect the number of casualties that results when a nuclear event is introduced.

## A. Radiation Incidents

According to the National Incident Management System (NIMS), an incident is “an occurrence, natural or manmade, that requires a response to protect life or property.”<sup>2</sup> To extrapolate from this definition, a radiation incident would be an occurrence that requires a response to protect life or property from exposure to or contamination from radiation. Medical countermeasures are one such response and could be used to prevent ARS from radiation exposure that occurs as a result of both radiation attacks and radiation accidents; both types of incidents can affect both civilian and military personnel.

Radiation attacks are the deliberate exposure to or contamination of people with radiation. These include the use of nuclear weapons, improvised nuclear devices, radiation dispersal devices, and radiation exposure devices. Nuclear weapons and improvised nuclear devices can result in widespread radiation casualties from prompt radiation and fallout, in addition to injuries incurred from blast and thermal burns. Radiation dispersal devices and radiation exposure devices, on the other hand, may result in some radiation exposure, though injuries from radiation would likely not be extensive. Also, contamination and injuries from the conventional explosion of a radiation dispersal device attack would not be likely in a radiation exposure device, since a radiation exposure device does not explode. Since nuclear weapons and improvised nuclear devices are the radiation incidents likely to result in the most casualties from IR, these are also the radiation scenarios in which radiation medical countermeasures may have the greatest benefit in lives saved. Therefore, in the illustrative example in which the fatalities from IR are estimated, fatalities from a nuclear weapon or improvised nuclear device detonation are the only radiation incidents considered.

Radiation accidents include radiation release from nuclear, industrial, or research facilities, incidents involving mishandling of orphan sources, or medical radiation misadministration. Examples of these include the Chernobyl nuclear reactor disaster

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<sup>1</sup> Although filgrastim is not FDA-approved for the treatment of ARS, it is approved for other indications, is in the Strategic National Stockpile (SNS), and has a demonstrated efficacy in treating ARS in non-human primates. See Chapter 3 for more information on filgrastim, its current regulatory status, and its mechanisms of action.

<sup>2</sup> Department of Homeland Security (DHS), *National Incident Management System* (Washington, DC: DHS, December, 2008).

(1986),<sup>3</sup> the contamination and exposure to Cesium-137 (Cs-137) in the city of Goiânia, Brazil (1987),<sup>4</sup> or the loss of an iridium-192 (Ir-192) source during the misadministration of radiotherapy to a patient in Indiana, Pennsylvania (1992).<sup>5</sup>

Radiation incidents of all these types could lead to IR doses great enough to cause ARS in those individuals exposed. While some incidents would likely cause more casualties from ARS than other incidents, medical countermeasures could potentially be used in all instances of ARS. Having FDA-approved medical countermeasures available for the treatment of ARS could assist in protecting life in the event of a radiation incident.

## B. Current and Future Acute Radiation Syndrome (ARS) Treatments

The human lethal dose of ionizing radiation for 50% ( $LD_{50/60}$ ) of those exposed is 450 centiGray (cGy). At the time of publication of this paper, there are no therapies approved by the FDA specifically for the treatment of ARS, meaning that in the absence of treatment half of people receiving 450 cGy would die. However, since ARS leads to a range of symptoms, such as emesis or fever, these symptoms and others can be treated with their respective FDA-approved therapies. The most significant research in radiation countermeasures has been in the implementation of supportive care, which includes a broad array of medical care options ranging from electrolyte and fluid replacement therapy, antibiotics and analgesics, and even stem cell replacement therapy. It is estimated that treatment of ARS patients with supportive care raises the  $LD_{50/60}$  to 675 cGy.<sup>6</sup>

Although it is not FDA-approved for ARS, the leukocyte growth factor filgrastim is stockpiled by the Centers for Disease Control and Prevention (CDC) in the Strategic National Stockpile (SNS), and the CDC holds an Emergency Use Investigational New Drug (IND) Application with the FDA for filgrastim administration for ARS.<sup>7</sup> If ARS is treated with supportive care and filgrastim, it is estimated that the human  $LD_{50/60}$  would increase to 850 cGy.<sup>8</sup> Other leukocyte growth factors, such as sargramostim are also

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<sup>3</sup> Chernobyl Forum, *Chernobyl's Legacy: Health, Environmental, and Socio-Economic Impacts and Recommendations to the Governments of Belarus, the Russian Federation and Ukraine* (Vienna, Austria: International Atomic Energy Agency (IAEA), 2006).

<sup>4</sup> IAEA, *The Radiological Accident in Goiânia* (Vienna, Austria: IAEA, 1988).

<sup>5</sup> IAEA, *Safety Reports Series No. 17: Lessons Learned from Accidental Exposures in Radiotherapy* (Vienna, Austria: IAEA, 2000).

<sup>6</sup> This  $LD_{50/60}$  is based on a dose reduction factor (DRF) of 1.5, which is estimated from a variety of sources. See Appendix B for details on how the DRF for supportive care was estimated.

<sup>7</sup> FDA, “Efficacy of Filgrastim in the Treatment of the Hematopoietic Syndrome of the Acute Radiation Syndrome Briefing Document,” presented at the Joint Meeting of the Medical Imaging Drugs Advisory Committee and Oncologic Drugs Advisory Committee, May 3, 2013, Pre-IND 100, 228.

<sup>8</sup> This  $LD_{50/60}$  is based on a DRF of 1.88, determined by T. J. MacVittie, A. M. Farese, W. Jackson III, “Defining the Full Therapeutic Potential of Recombinant Growth Factors in the Post Radiation-

procured in the SNS.<sup>9</sup> Based on the outcome of the FDA Joint Meeting of the Medical Imaging Drugs Advisory Committee and the Oncologic Drugs Advisory Committee, companies that market leukocyte growth factors can submit supplemental biological licensure applications (sBLA) for a similar Emergency Use IND for a radiation incident.<sup>10</sup> Filgrastim, sargramostim, and other leukocyte growth factors are discussed in Chapter 3. Therefore, there exist several potential therapies, which are not FDA-approved for ARS and have other indications that could be used to treat ARS in an emergency. Their ultimate FDA approvals for ARS are being pursued, and it seems unlikely that any extra DOD investment in research here will prove advantageous.

Funding for R&D of future therapies can be approached in various ways, and not all approaches require the traditional pharmaceutical model of bringing a new molecule or biologic to market *de novo*, that is from basic research through pre-clinical and all safety and efficacy trials. This traditional drug development approach costs around 1.3 billion in 2005 United States Dollars (USD),<sup>11</sup> and a recent Forbes magazine estimate places current estimates ranging from a mean cost of between 1 and 6 billion 2013 USD.<sup>12</sup> This traditional R&D approach is about 10 to 15 years in duration from drug target selection to FDA approval.<sup>13</sup> Furthermore, of drugs that enter Phase I clinical trials, it is estimated that only 7.5% actually reach approval.<sup>14</sup> Clearly these billions of dollars that could be spent in traditional drug development would have a very low likelihood of success, and since the radiation therapies require a nontraditional path for testing efficacy—the Animal Rule—the likelihood of success is even lower. This traditional pharmaceutical R&D approach is disadvantageous based on cost, risk, and time to the actual fielding of a radiation therapy.

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Accident Environment: The Effect of Supportive Care Plus Administration of G-CSF,” *Health Physics* 89 (2005): 546–55.

<sup>9</sup> Department of Health and Human Services, “HHS Boosts Stockpile of Products to Treat Acute Radiation Syndrome,” news release dated September 26, 2013, last revised September 26, 2013, last accessed June 13, 2014, <http://www.hhs.gov/news/press/2013pres/09/20130926a.html>.

<sup>10</sup> FDA, Center for Drug Evaluation and Research, “Summary Minutes of the Joint Meeting of the Medical Imaging Drugs Advisory Committee and the Oncologic Drugs Advisory Committee,” May 3, 2013.

<sup>11</sup> J. A. DiMasi and H. G. Grabowski, “The Cost of Biopharmaceutical R&D: Is Biotech Different?” *Managerial and Decision Economics* 28 (2007): 469–79.

<sup>12</sup> M. Herper, “The Cost of Creating a New Drug Now \$5 Billion, Pushing Big Pharma to Change,” *Forbes* August 11, 2013, last accessed August 22, 2014, <http://www.forbes.com/sites/matthewherper/2013/08/11/how-the-staggering-cost-of-inventing-new-drugs-is-shaping-the-future-of-medicine/>.

<sup>13</sup> S. M. Paul et al., “How to Improve R&D Productivity: The Pharmaceutical Industry’s Grand Challenge,” *Nature Reviews Drug Discovery* 9 (2010): 203–14.

<sup>14</sup> For new molecular entities, from M. Hay et al., “Clinical Development Success Rates for Investigational Drugs,” *Nature Biotechnology* 32 (2014): 40–51.

However, the traditional R&D approach is not the only way available to DOD, the U.S. Government (USG), academia, or the private sector to achieve a fielded therapy. Multiple other drug development paradigms exist, and some are being explored in earnest today because the cost, risk, and time to bring a therapy to approval *de novo* are so great. In this paper some of these alternate drug development paradigms are explored, including repurposing pharmaceuticals and biologics and testing combinations of therapies. Furthermore, DOD can consider alternate research avenues, which are especially important in the preliminary basic research required for finding countermeasures that could protect against the high IR doses that cause GI ARS. These types of investments include creating dual funding sources that combine DOD funding with other funding for therapies that treat disorders with mechanistic overlap to the gastrointestinal (GI) subsyndrome. Also, DOD could support the creation of applied research tools such as high throughput screen (HTS) assays, which could hasten the discovery of new drugs with novel mechanisms, especially those that target GI ARS. Lastly, in order to increase the likelihood that an ARS countermeasure would be approved by the FDA, the authors recommend that DOD support the development of an FDA-approved animal model to establish the efficacy of all ARS countermeasures.

Alternatives to traditional drug development are described in detail in Chapter 2, followed by some example potential countermeasures in Chapter 3, most of which do not offer much protection beyond supportive care and filgrastim. Chapter 4 shows an illustrative example of the use of supportive care and filgrastim in nuclear environments and how the number of fatalities changes with their implementation. Chapter 5 gives the IDA research team's recommendations for ARS countermeasure strategies.



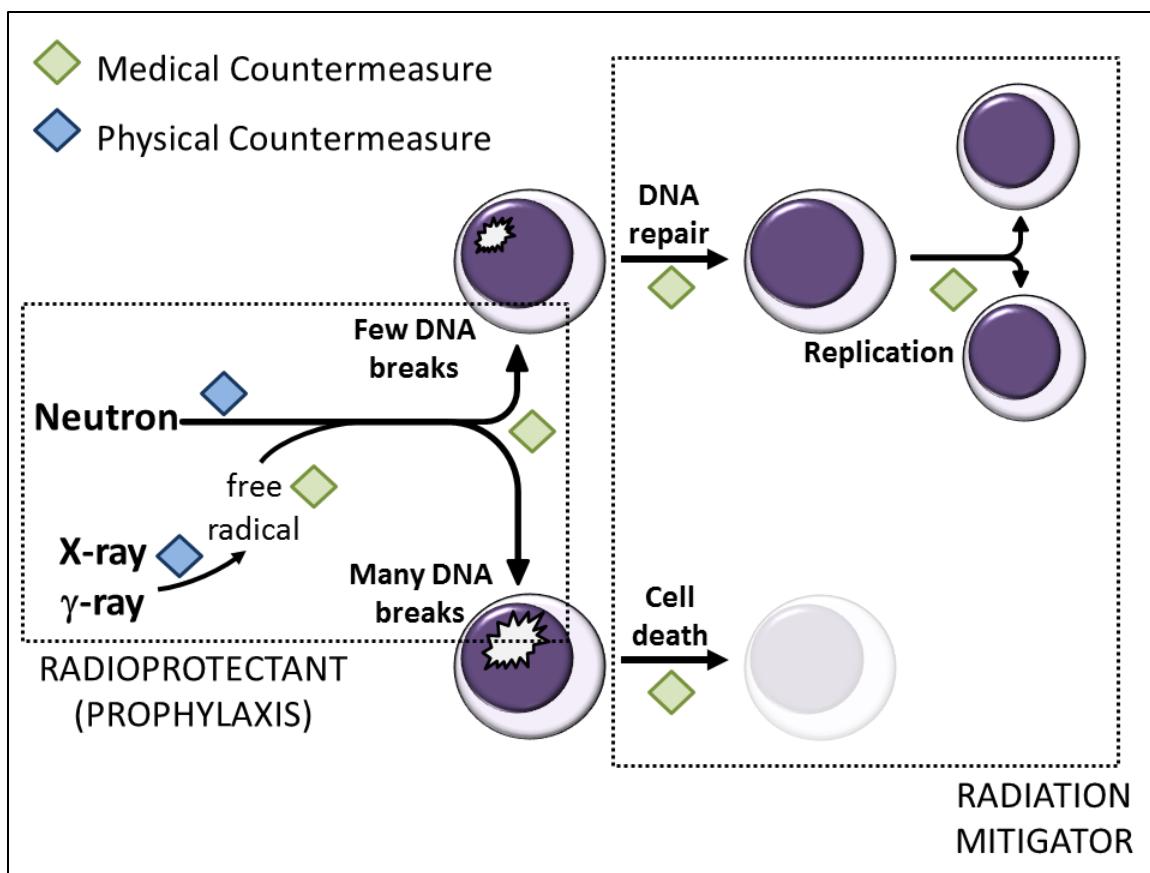
## **2. Drug Development Strategies for Medical Countermeasures against Ionizing Radiation**

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Medical countermeasures to ameliorate the human response to IR would apply both to radiological and nuclear events. Two broad categories of radiation medical countermeasures exist based on their respective timings of intervention. These two categories are radioprotectants and radiation mitigators (Figure 1).<sup>15</sup> More information on how IR damages DNA, how cells respond to this damage, and the pathogenesis of ARS can be found in Appendix A.

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<sup>15</sup> Physical countermeasures, such as lead shielding that would prevent IR from ever reaching the cells, also exist but are not discussed here.



**Figure 1. Potential Points in the IR-Damage Pathway of Cells for Medical Countermeasures against ARS**

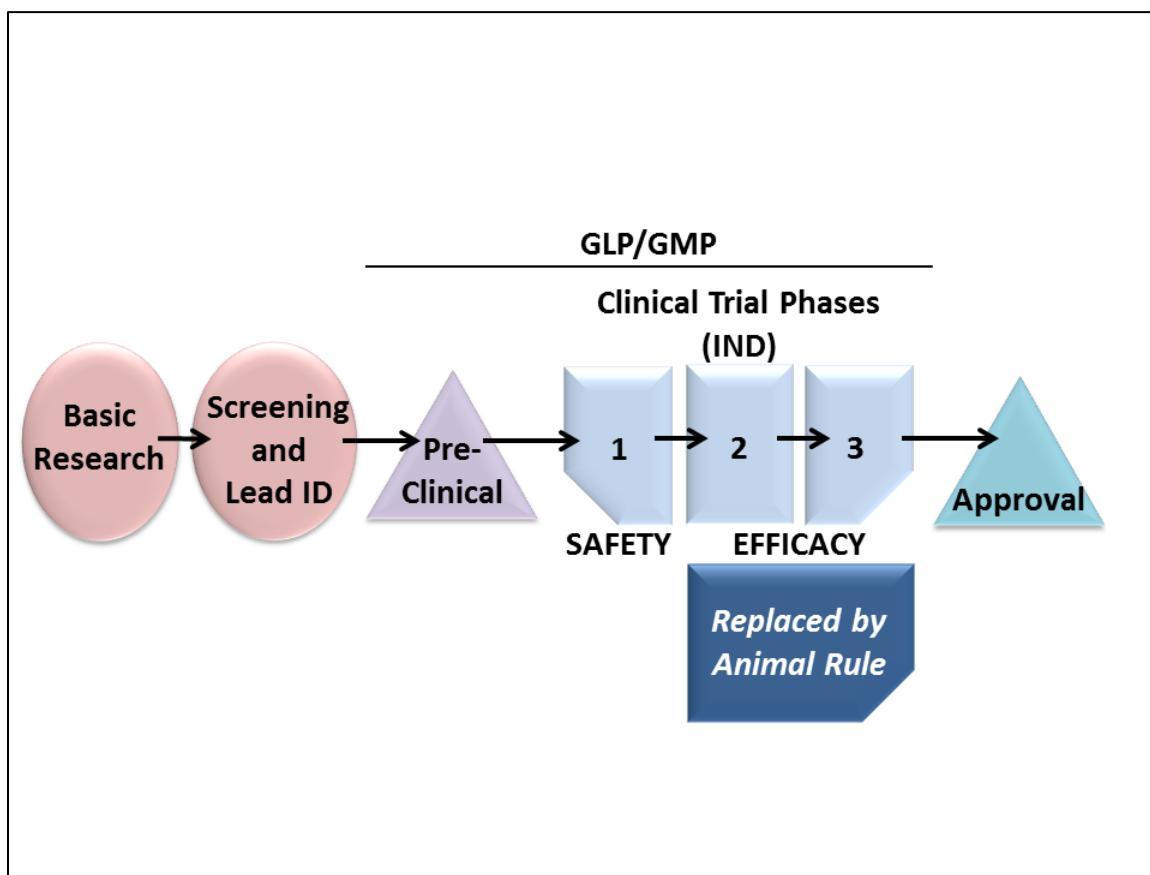
Radioprotectants would be given as a prophylaxis since they must be present in the body at the time of IR exposure. Potential radioprotectants would be free radical scavengers or therapies that could prevent or decrease the number of DNA strand breaks. In Figure 1, this type of countermeasure would shift the number of breaks to the “Few DNA breaks” path from which the cell could more likely recover. Of the two points of intervention shown for radioprotectants, only free radical scavengers have been explored in any significant way as therapies.

Radiation mitigators would be administered post-IR exposure, since their activities assist the cell in recovering from the insult. The time after exposure at which the mitigator could be used would be dependent on the individual therapy. Mitigators have the potential to assist in DNA repair, enhance the recovered cells’ abilities to proliferate and repopulate, or inhibit cell death, which could allow these cells to serve as sources of stem cells for repopulation. Of these three points of intervention, only therapies that enhance cell replication have been significantly studied.

In the following sections of this chapter, an overview of the FDA approval process and the traditional drug development model is provided. Other models of drug development and additional research avenues will also be explored.

## A. United States (U.S.) Food and Drug Administration (FDA) Approval of New Drugs or Biologics to Treat Chemical, Biological, Radiological, and Nuclear (CBRN) Injuries

Approval of a new drug requires submission of an IND to the FDA. The two key components of an IND are the demonstrations of human safety and human efficacy. These include Phases 1, 2, and 3 of clinical drug trials, with Phase 1 focusing on safety and Phases 2 and 3 on efficacy. All phases require prior and current demonstration of and adherence to good laboratory practice (GLP) and good manufactory practice (GMP). GLP and GMP have to be established in pre-clinical research. Prior to pre-clinical research is work that can include basic research, screening of compounds, and lead compound selection. Figure 2 describes this process.



**Figure 2. The Process of Drug Discovery and Approval, Including the Replacement of Phases 2 and 3 by the Animal Rule**

Like all therapies, the approval of Chemical, Biological, Radiological, and Nuclear (CBRN) countermeasures requires the demonstration of both safety and efficacy, and Phase 1 safety trials are similar to other INDs. Human safety studies in healthy volunteers are usually possible for most CBRN treatments, as the

FDA's procedures and standards for evaluating the safety of new drug and biological products are sufficiently flexible to provide for the safety evaluation of products evaluated for efficacy under subpart I of part 314 and subpart H of part 601.<sup>16</sup>

However, the efficacy of CBRN countermeasures cannot be demonstrated in humans, and Phase 2 and 3 clinical trials are replaced by the FDA Animal Rule (Figure 2). Initially published in 2002, the Animal Rule allows an avenue for the approval of drugs for the purpose of "ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic chemical, biological, radiological, or nuclear substances"<sup>17</sup> that were outside of the normal FDA clinical trial approval process, since "it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance."<sup>18</sup> Most INDs fail to be approved because they do not show sufficient efficacy.<sup>19</sup> While the Animal Rule has not been established for long enough to have gathered sufficient data on IND approval rates, it would be expected that the approval rate would be even smaller for CBRN countermeasures than other pharmaceuticals, since the nuances of how the Animal Rule will function have not been fully determined.

## B. Identification of a Qualified Animal Model for ARS: A Prerequisite for Countermeasure Approval

No ARS medical countermeasure can be approved until the FDA deems a specific animal model to be an appropriate surrogate of ARS in humans. Therefore, identification of a qualified animal model for ARS should be a priority for ARS countermeasure research. A qualified animal model need not be established or researched in isolation. When the National Institute for Allergies and Infectious Diseases (NIAID) designed the non-human primate (NHP) study for their submission to the FDA for filgrastim indication for ARS, they sought FDA guidance,<sup>20</sup> and the suitability of their NHP model for ARS

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<sup>16</sup> "New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible," Final Rule, *Federal Register* 67 (2002): 37988–98.

<sup>17</sup> FDA, "Applications for FDA Approval to Market a New Drug," Title 21, Code of Federal Regulations (CFR) 314 (Washington, DC: U.S. Department of Health and Human Services (DHHS), revised April 1, 2013).

<sup>18</sup> Ibid.

<sup>19</sup> Hay et al., "Clinical Development Success Rates for Investigational Drugs," 40–51.

<sup>20</sup> FDA, "Safety and Efficacy of Currently Approved Leukocyte Growth Factors (LGFs) as Potential Treatments for Radiation-induced Myelosuppression Associated with a Radiological/Nuclear Incident," FDA Advisory Committee Briefing Document for Joint Meeting of the Medical Imaging Drugs Advisory Committee and the Oncologic Drugs Advisory Committee, 2013.

injury in humans was one of the primary topics for consideration when the efficacy of filgrastim as an ARS countermeasure was evaluated.

In the Joint Meeting of the Medical Imaging Drugs Advisory Committee and the Oncologic Drugs Advisory Committee on May 3, 2013, the NIAID NHP study was evaluated. The goal was to move filgrastim toward FDA indication for treating ARS and to understand the extent to which this work could be extrapolated to other leukocyte growth factors, namely sargramostim, pegfilgrastim, and tbo-filgrastim. The committee voted 17 to 1 that filgrastim was “reasonably likely to produce clinical benefits in humans exposed to radiation that is likely to induce myelosuppression during or following a radiological/nuclear event.” The one dissenting individual did not believe that this type of incident would produce radiation doses great enough to induce myelosuppression. Some of the other considerations that the committee grappled with centered on their desire to see more data on filgrastim administration at different IR doses, different times post-IR, and the difference between the neutrophil repopulation kinetics in humans and NHPs.

A second animal model has been implemented by the company Neumedicines, Inc in its NHP studies of the efficacy of recombinant human interleukin-12 (rHuIL-12) to treat hematopoietic (HP) ARS. This model does not use most supportive care measures including antibiotics, intravenous (IV) fluids, and blood transfusions. This model does allow painkiller administration, treatment of mouth ulcers, and Pepto-Bismol.<sup>21</sup> The company’s reasoning for excluding most supportive care measures is that in a mass casualty scenario, it would be difficult to distribute medical care to most people. While rHuIL-12 does show a two-fold increase in survival at an LD<sub>90/60</sub> IR dose in this NHP model, it is unclear whether the FDA would support this as a qualified animal model. While it may be more reflective of the scenario that Neumedicines suggests following a radiation incident, the studies were performed outside the United States, and there is disagreement among researchers regarding the minimum of supportive care for NHPs that is considered ethical. To IDA’s knowledge, this work has not yet been reviewed by the FDA, and Neumedicines states that in part with funding from Biological Advanced Research and Development Authority (BARDA) that Neumedicines plans to apply for Emergency Use Authorization and FDA licensure of rHuIL-12 in 2015 and 2016, respectively.<sup>22</sup> It is possible that both this NHP model and the model used in the filgrastim experiments discussed earlier would be acceptable to the FDA.

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<sup>21</sup> Z. Gluzman-Poltorak, S.R. Mendonca, V. Vainstein, H. Kha, L.A. Basile, “Randomized Comparison of Single Dose of Recombinant Human IL-12 versus Placebo for Restoration of Hematopoiesis and Improved Survival in Rhesus Monkeys Exposed to Lethal Radiation,” *Journal of Hematology and Oncology* 7, no.31 (2014).

<sup>22</sup> “BARDA Awards 14M for Acute Radiation Countermeasure,” *Global Biodefense*, August 18, 2014, last accessed September 2, 2014, <http://globalbiodefense.com/2014/08/18/barda-awards-14m-acute-radiation-countermeasure/>.

The FDA recently introduced its Animal Model Qualification Program (AMQP) through its Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER) to provide a mechanism by which an animal model can be considered acceptable by the FDA as a model for efficacy testing in countermeasure development. The AMPQ also shows that the FDA recognizes the difficulty in complying with the Animal Rule and that the FDA hopes to provide a mechanism to streamline approval for CBRN countermeasures. An animal model will be qualified for a specific context of use (COU), which includes

characterization of the animals to be used, characterization and preparation of the challenge agent, procedural information for the challenge agent exposure, identification of the primary and secondary endpoints, triggers for intervention, and ranges of values of key parameters of the disease or condition that will be used as measures of quality control and quality assurance when the model is replicated.<sup>23</sup>

While the FDA states that qualification of an animal model is “not required for product approval or licensure under the Animal Rule,”<sup>24</sup> establishing a qualified model for ARS is the first major step in truly advancing ARS countermeasure research. A qualified model would include primary and secondary endpoints, which could be directly compared among treatments. It would also standardize confounding factors such as supportive care, which is currently implemented differently in various countermeasure animal studies. In short, performing ARS countermeasure research in a qualified animal model could lead to approval of treatments as well as direct efficacy comparisons between putative countermeasures.

### C. Alternate Drug Development Strategies for ARS Countermeasures

The traditional drug development paradigm begins with selecting a target compound that shows promise as a therapy for a specific disease. This selection is usually performed in assays that do not require complex animal models. As a result the cost per experiment is less but the results are less likely to be applicable to humans. Following target selection, pre-clinical trials are performed, followed by Phase 1 safety trials and Phase 2 and 3 efficacy trials. This process is costly (1 to 6 billion USD<sup>25</sup>), long (10 to 15 years<sup>26</sup>), and has a low chance of resulting in an approved therapy (7.5% of new molecular entities

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<sup>23</sup> FDA, “Animal Model Qualification Program,” last updated April 15, 2014. <http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm284078.htm>.

<sup>24</sup> Ibid.

<sup>25</sup> Herper, “The Cost of Creating a New Drug Now \$5 Billion, Pushing Big Pharma to Change.”

<sup>26</sup> Paul et al., “How to Improve R&D Productivity: The Pharmaceutical Industry’s Grand Challenge,” 203–14.

that enter Phase 1 clinical trials are approved<sup>27</sup>). These estimates are likely greater for ARS therapies, since they require the Animal Rule for efficacy studies.

Since current candidate ARS therapies show little ability to treat beyond two LD<sub>50</sub>s IR and the risk, time, and cost to development are so great, it is unsurprising that drug companies and stakeholders alike shy away from investing much capital in their development. However, the traditional drug development strategy is not the only available paradigm, and alternate strategies are becoming more commonplace in the pharmaceutical industry, academia, and government. Two of these alternate strategies, drug repurposing and combination therapies, are discussed below.

## **1. Repurposing/Repositioning Drugs for ARS Countermeasures**

In contrast to the traditional drug development model, repurposing of previously FDA-approved drugs for a new indication, such as ARS, could cut cost, time to approval, and risk. Since these drugs already have established safety profiles for their previous indications, the burden to establish safety, perhaps at a different dose required for the new indication, is reduced. Some FDA-approved drugs may be indicated for conditions similar to ARS, and these data are considered to support new, similar indications.

Drug repurposing is not a novelty; examples of alternate indications include the use of the analgesic aspirin to treat cardiovascular disease and the repurposing of the sedative thalidomide to treat leprosy.<sup>28</sup> Recently, however, drug repurposing has gained popularity among pharmaceutical companies and translational researchers. The National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH) has an entire program centered on repurposing drugs, including partnerships with large pharmaceutical companies such as Eli Lilly to further their discovery of novel targets for previously indicated drugs.<sup>29</sup>

The Institute of Medicine (IOM) focused on drug repurposing in its recent 2013 Roundtable on Translating Genomic-Based Research for Health. Their 2014 report provides an excellent summary of considerations, advantages, and disadvantages for using the repurposing strategy for drug development. Topics discussed include the

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<sup>27</sup> Hay et al., “Clinical Development Success Rates for Investigational Drugs,” 40–51.

<sup>28</sup> J. K. Aronson, “Old Drugs—New Uses,” *British Journal of Clinical Pharmacology* 64 (2007): 563–5.

<sup>29</sup> National Center for Advancing Translational Sciences, “Discovering New Therapeutic Uses for Existing Molecules,” last accessed September 4, 2014, <http://www.ncats.nih.gov/research/reengineering/rescue-repurpose/therapeutic-uses/therapeutic-uses.html>.

reduction in cost, time to approval, and risk of approval compared to traditional drug development.<sup>30</sup>

The workshop estimated that the cost of repurposing a drug is 60% of the cost of traditional drug development. The time to approval can be as short as 3 years versus 15 years for traditional drug development. Furthermore, the risk of approval is much reduced for repurposed drugs, with estimates of 30% (versus 7.5%) of repurposed drugs achieving FDA approval.<sup>31</sup> Drug repurposing provides a significant savings over traditional drug development.

## 2. Combination Therapies with Other Indications Codeveloped to Treat ARS

Complex diseases often have multiple biological pathways that underlie the sequelae. Multiple pathways suggest that multiple drug targets may exist, and treating multiple components of a disease can lead to greater efficacy. Combination therapy is a strategy that has traditionally been focused on cancer and infectious disease, but there is now interest in its application to other diseases such as neurological and autoimmune disorders.<sup>32</sup>

ARS is incredibly complex and its complexity seems to increase with dose due to the appearance of the GI and cerebrovascular (CV) subsyndromes, and it is well established that multiple biological disturbances occur from IR exposure that lead to ARS. Multiple symptom treatments are already combined in supportive care therapy for ARS, but this drug development strategy suggests pursuit of the codevelopment of drugs for the treatment of ARS. For example, a leukocyte growth factor used in conjunction with an inflammatory bowel disorder drug might be more effective in treating GI ARS than one of these drugs alone.<sup>33</sup>

While using therapies in concert holds promise for novel treatments of complex diseases, it also raises new pharmacological and regulatory challenges. The FDA recognizes the potential benefit of drug codevelopment, and it addresses the regulatory and scientific issues in its “Guidance for Industry: Codevelopment of Two or More New Investigational Drugs for Use in Combination.”<sup>34</sup>

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<sup>30</sup> IOM, *Drug Repurposing and Repositioning: Workshop Summary* (Washington, DC: National Academies Press, 2014).

<sup>31</sup> Ibid.

<sup>32</sup> J. Woodcock, J. P. Griffin, and R. E. Behrman, “Development of Novel Combination Therapies,” *New England J Medicine* 364 (2011): 985–7.

<sup>33</sup> This example is purely notional.

<sup>34</sup> FDA, “Guidance for Industry: Codevelopment of Two or More New Investigational Drugs for Use in Combination,” June 2013.

New screening technology and other scientific advances have been pioneered by startup biotechnology companies that focus on combination therapies. Considering the progression both in regulation and strategies for codevelopment of drugs, this may be an avenue for DOD to consider especially with the goal of finding ARS therapies that can treat doses of IR much greater than two LD<sub>50</sub>s.

## **D. Alternate Research Avenues to Complement Current Drug Development Efforts**

### **1. Supporting Drug Development of Therapies for Treatment of Gastrointestinal (GI) Disorders with Similar Mechanisms to GI ARS**

The understanding of the gut stem cell and epithelial cell repopulation dynamics remains nascent compared to scientific knowledge of hematopoiesis. However, significant mechanistic overlap is becoming apparent between the pathologies of inflammatory bowel diseases (IBD) and that of GI ARS.<sup>35</sup> Supporting drug development and basic research into IBD as well as therapies that could protect against intensive chemoradiotherapy of gut tissue could bring DOD closer to a therapy that would be effective against GI ARS. Similar to the collaboration between DOD, other government entities, and bone marrow transplant and cancer centers to further radiation injury treatment, DOD could collaborate with other government or non-government funding entities to further the understanding of both non-ARS GI disorders and GI ARS.<sup>36</sup>

### **2. Assay Development for New Candidate Drug Identification**

Drug candidate selection for development of ARS treatments typically uses a biased approach in which a specific candidate compound is first selected, for various reasons, and then its efficacy in treating ARS is tested in an ARS animal model. While this can lead to new treatment candidates, it excludes the possibility of using one or more of the thousands to millions of other compounds to treat ARS. Rapid, high-throughput screens (HTS) are utilized in early candidate screening for various diseases. Libraries at various

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<sup>35</sup> J. Kountouras and C. Zavos, “Recent Advances in the Management of Radiation Colitis,” *World J Gastroenterology* 14 (2008): 7289–7301; C. R. Camara-Lemarroy, “Remote Ischemic Preconditioning as Treatment for Non-Ischemic Gastrointestinal Disorders: Beyond Ischemia-Reperfusion Injury,” *World J Gastroenterology* 20 (2014): 3572–81; and A. V. K. Indaram, V. Visvalingam, M. Locke, and S. Bank, “Mucosal Cytokine Production in Radiation-induced Proctosigmoiditis Compared with Inflammatory Bowel Disease: Mucosal Cytokines in Radiation Proctitis,” *Am J Gastroenterology* 95 (2000): 1221–5.

<sup>36</sup> Some example funding sources supporting GI research include the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute for Allergies and Infectious Diseases (NIAID), National Cancer Institute (NCI), Crohn’s and Colitis Foundation of America (CCFA), and Kenneth Rainin Foundation.

institutions can be applied to these HTS to discern candidate molecules for follow-up research. HTS can be effective tools for new compound identification, as well as for combinatorial chemistry to identify more effective variants of a lead compound.

To maximize the utility of HTS, the assay used must be representative of some important druggable pathway of the disease state. In the case of GI ARS, for example, an assay that mimics intestinal stem cell regeneration or protects gut cells from inflammatory molecules associated with ARS could be used to screen the NIH Molecular Libraries Small Molecule Repository of over 300,000 potential therapeutic compounds. If compounds with known human safety profiles are desired, the NIH Clinical Collection of about 450 small molecules could be screened. Based on results from HTS, candidate molecules could be investigated in more specific assays and eventually in animal models for ARS.

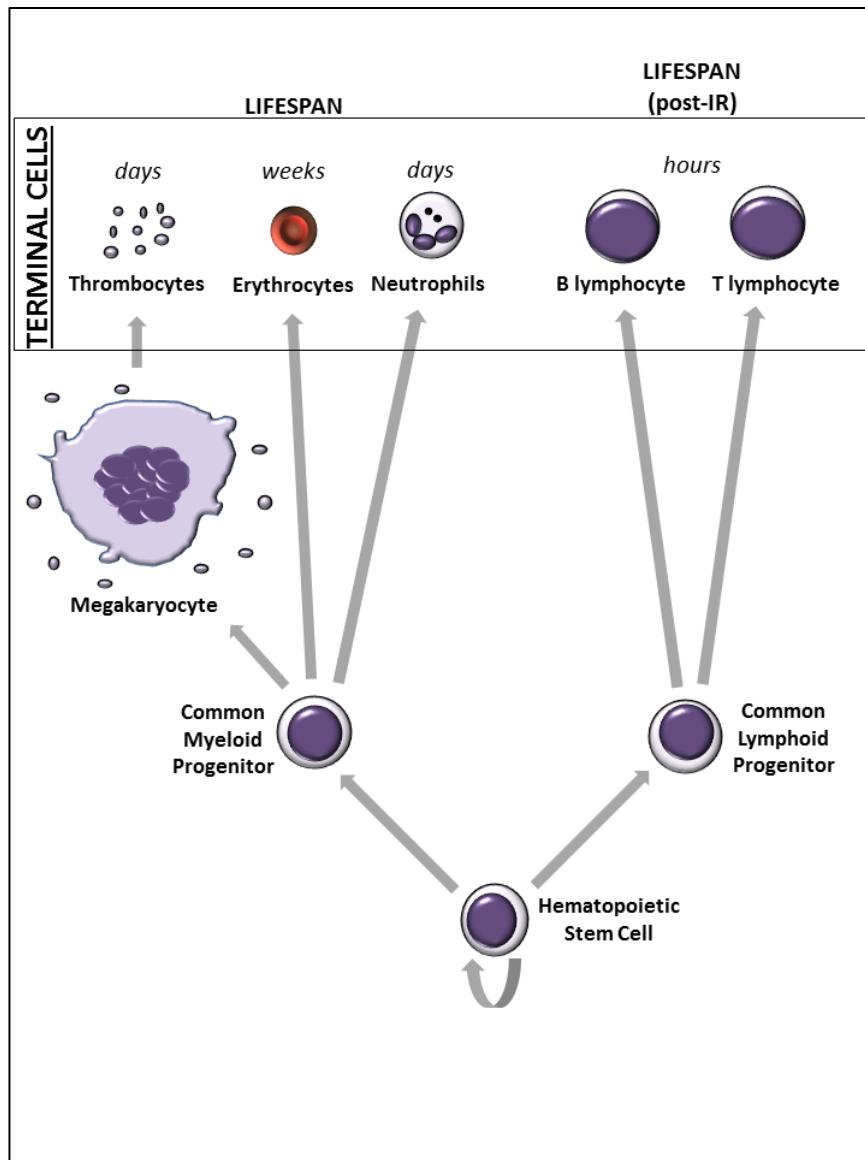
Since there are currently no candidate ARS countermeasures that are effective at protecting against IR doses greater than about two LD<sub>50</sub>s, novel compounds with potentially unexpected targets might prove useful in the discovery of more effective ARS therapies. DOD could consider smaller investments in HTS assay development for screening compound libraries, as well as supporting the follow-up research that could result from such efforts.

### **3. Experimental Medical Countermeasures for ARS**

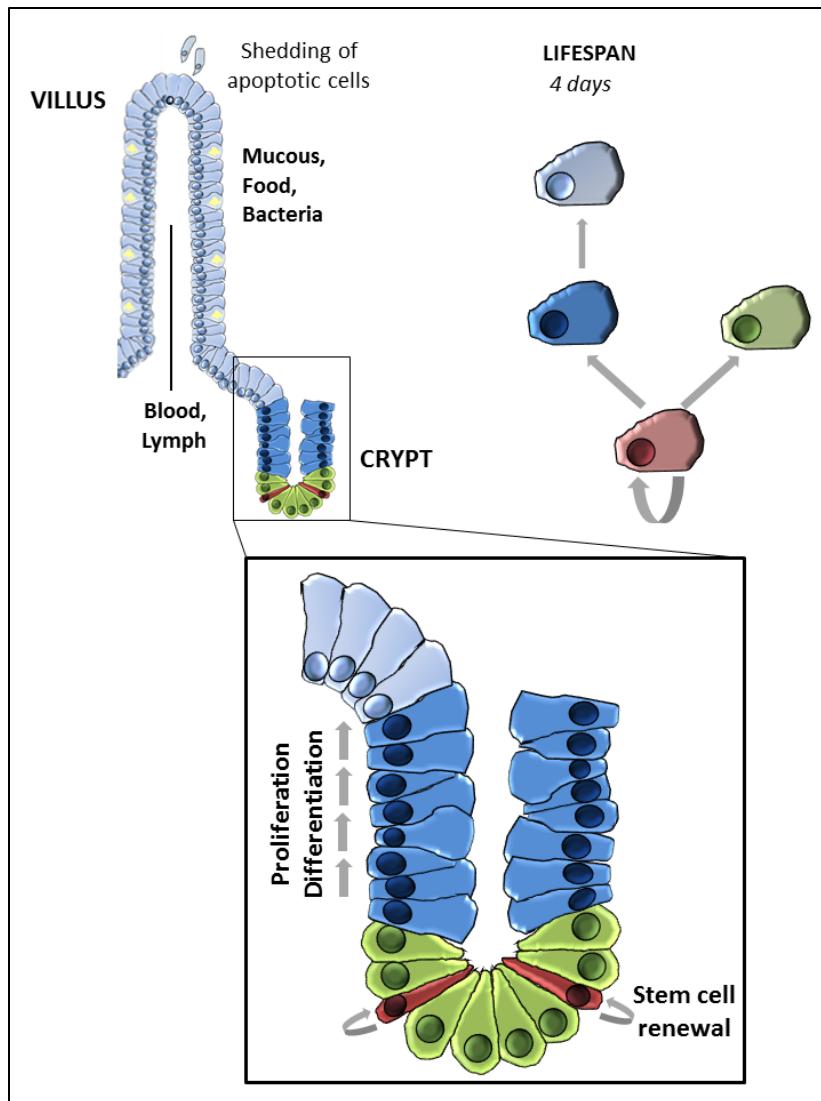
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Prospective countermeasures with many types of data supporting their varying potential to treat ARS are described next. Since the demonstrations of safety and efficacy are the cornerstones of bringing a countermeasure to approval by the FDA, the quality and quantity of both of these types of experiments are discussed, as well as the strategies—traditional or repurposing—that are being used to develop these drugs as ARS therapies. In several instances, various potential countermeasures have been used to treat ARS following worldwide radiological accidents. Unfortunately, these do not provide much data about their efficacies or safeties, since these instances provide no controls to which to compare them. Instances of their use, however, are noted where applicable.

The authors assume that the reader has a general understanding of ARS and the underlying cellular damage that results from IR exposure. Both the HP and GI systems are primary targets of IR damage. Normal repopulation of the HP and GI systems, as well as the key stem cell and terminal cell components, are shown in Figure 3 and Figure 4 for reference. Further review can be found in Appendix A.



**Figure 3. Hematopoiesis Showing HP Stem Cells Giving Rise to Progenitors which Further Differentiate into Terminally Differentiated Cells**



**Figure 4. GI Epithelial Cell Populations and Their Turnover in the Intestines**

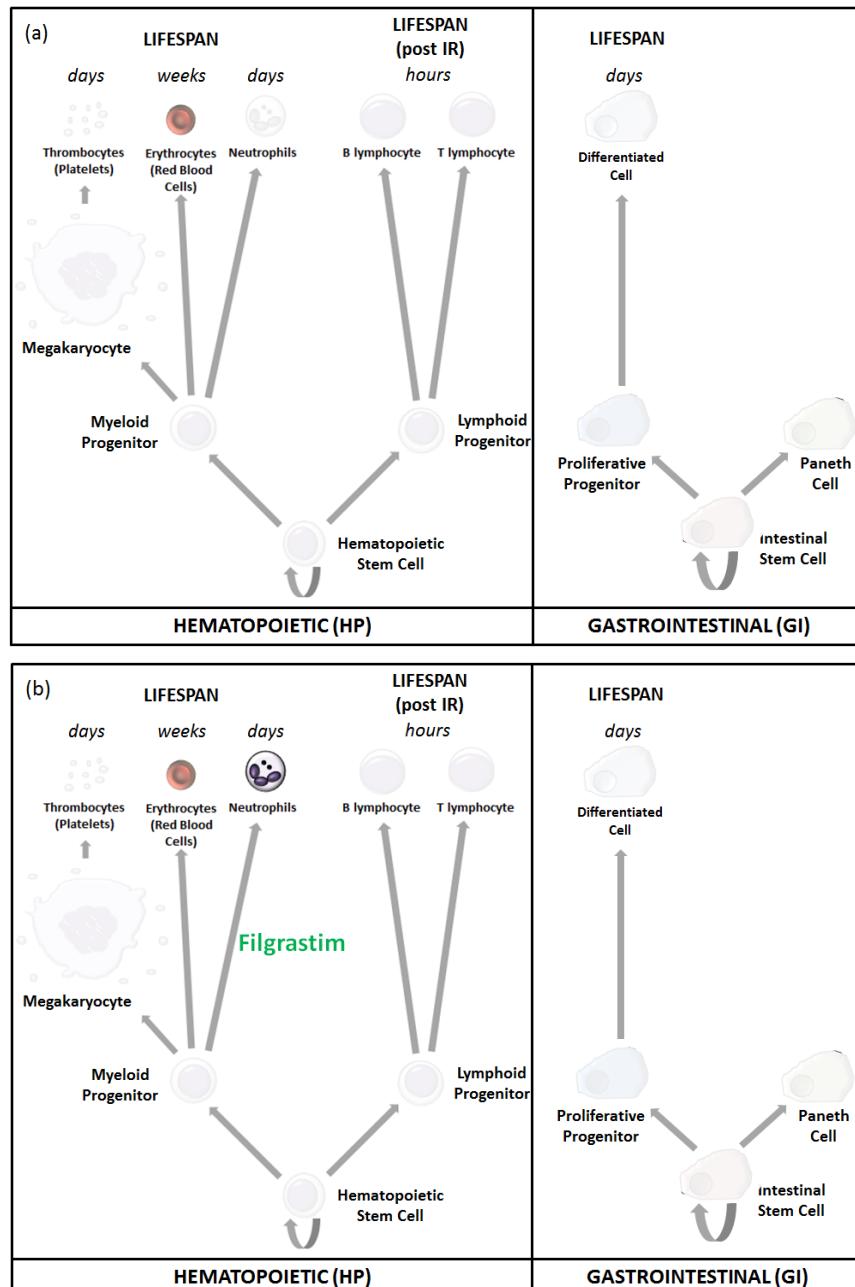
## A. Leukocyte Growth Factors for the Treatment of ARS

Both filgrastim and sargramostim have extensive safety and efficacy data. While they are not FDA-approved for the treatment of ARS, the CDC is stockpiling both Neupogen® (filgrastim) and Leukine® (sargramostim) for use in a radiological or nuclear emergency.

### 1. Filgrastim and Pegfilgrastim

Filgrastim is a synthetic human granulocyte colony-stimulating factor (G-CSF) produced in *Escherichia coli* (*E. coli*) using recombinant DNA technology. Biosimilars of filgrastim are available from numerous companies. It is stored under refrigeration and

can be injected subcutaneously. Filgrastim is similar to the protein with the same name made in human cells, with a few changes to allow for its efficient isolation from *E. coli*. In the body, G-CSF is important in mobilizing and maturing HP stem cells into neutrophils (Figure 5).



Note: (a) Without filgrastim, IR depletes terminal and stem cells, and (b) With filgrastim treatment the neutrophil nadir is not as severe and the neutrophils recover more quickly.

**Figure 5. Filgrastim Administration Assists the Bone Marrow in Repopulating Neutrophils**

Although endogenous G-CSF is also produced following IR exposure,<sup>37</sup> the exact source of the G-CSF in the body following IR is not known. G-CSF is documented to be released, leading to an increase in circulating neutrophils, following infection and environmental stress, though this response varies based on the stress or infectious agent. There is evidence that the endogenous G-CSF increases myeloid lineage commitment, bone marrow mobilization, and neutrophil survival and proliferation.<sup>38</sup> In several radiation countermeasures still in discovery, it has been shown that the survival effects of the countermeasures are mediated, at least in part, by stimulating endogenous G-CSF.<sup>39</sup>

#### a. Comparison of Filgrastim and Pegfilgrastim

Pegfilgrastim is a modified version of G-CSF with a polyethylene glycol (PEG) moiety covalently attached. The major effect of pegylation on filgrastim in the body is on its clearance. The mean elimination half-life is approximately 3.5 hours for filgrastim.<sup>40</sup> The elimination of pegfilgrastim is directly proportional to neutrophil levels, and it ranges from 15 to 80 hours.<sup>41</sup> Like filgrastim, pegfilgrastim is stored under refrigeration and can be injected subcutaneously. To prevent neutropenia in patients receiving myeloablative chemotherapy, it is recommended that the first dose of filgrastim be administered 24 to 72 hours after chemotherapy, though filgrastim is recommended to be administered up to 120 hours following high-dose chemotherapy. Unlike filgrastim, which requires repeated doses, pegfilgrastim is given just once, 24 hours after chemotherapy is completed. To note, filgrastim can be used in pediatric populations<sup>42</sup> and is recommended,<sup>43</sup> whereas pegfilgrastim is not currently recommended for use in pediatric patients,<sup>44</sup> though clinical trials are underway.<sup>45</sup>

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<sup>37</sup> V. K. Singh et al., “Role of Radiation-induced Granulocyte Colony-stimulating Factor in Recovery from Whole Body Gamma-irradiation,” *Cytokine* 58 (2012): 406–14.

<sup>38</sup> D. C. Link, “Mouse Models of G-CSF Signaling in Hematopoiesis,” in *Twenty Years of G-CSF: Clinical and Nonclinical Discoveries*, ed. G. Molineux, M. Foote, and T. Arvedson (Basel: Springer, 2012).

<sup>39</sup> V. K. Singh et al., “A Review of Radiation Countermeasure Work Ongoing at the Armed Forces Radiobiology Research Institute,” *Int J Radiat Biol* 88 (2012): 296–310.

<sup>40</sup> Neupogen® (filgrastim) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2012).

<sup>41</sup> Neulasta® (pegfilgrastim) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2012).

<sup>42</sup> Neupogen® (filgrastim) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2012).

<sup>43</sup> T. J. Smith et al., “2006 Update of Recommendations for the Use of White Blood Cell Growth Factors: An Evidence-based Clinical Practice Guideline,” *J Clin Oncol* 24 (2006): 3187–205.

<sup>44</sup> Neulasta® (pegfilgrastim) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2012); Smith et al., “2006 Update of Recommendations for the Use of White Blood Cell Growth

### **b. Human Safety of Filgrastim and Pegfilgrastim**

Filgrastim and pegfilgrastim are both considered safe in humans at doses that would be used to treat ARS. The most common use of these drugs is for chemotherapy-induced neutropenia, for which they are both FDA-approved and have been used extensively. One side effect of cancer treatment is neutropenia, and if neutropenia becomes too severe, the chemotherapy schedule is altered to allow recovery. Use of filgrastim and pegfilgrastim is commonplace, allowing patients to remain on their treatment schedules and resulting in improved prognoses. Filgrastim, but not pegfilgrastim, is also indicated for peripheral blood progenitor cell mobilization.<sup>46</sup>

Cytokine therapy has already been used off-label following radiation incidents. Dainiak et al. describe eight patients who received filgrastim in response to IR exposure, including at the Tokai-mura nuclear criticality accident, Tokai-mura, Japan (1999)<sup>47</sup>; Gilan, Iran accident (1996)<sup>48</sup>; and the Istanbul, Turkey accident (1998).<sup>49</sup> As a result of reviewing these cases and animal studies, the authors recommended that filgrastim be used in clinical profiles of ARS that suggested it would improve prognosis.<sup>50</sup> Other instances where G-CSF has been prescribed in response to exposure to IR are Yanango, Peru (1999)<sup>51</sup> and Samat Prakarn Province, Thailand (2000).<sup>52</sup>

### **c. Efficacy of Filgrastim in Treating ARS**

While filgrastim is prescribed in the clinic for neutropenia in addition to the few instances of its use off-label for ARS, its approval for ARS is being actively pursued. In May 2013, the pre-IND briefing was presented to the FDA, including data on the use of

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Factors,” 3187–205; and Amgen, Neulasta® (pegfilgrastim), Oncologic Drugs Advisory Committee Pediatric Subcommittee Meeting, FDA, 20 October 2005.

<sup>45</sup> ClinicalTrials.gov, “NCT00035620: Pegfilgrastim as Support to Pediatric Sarcoma Patients Receiving Chemotherapy,” Verification date: February 2010,  
<http://clinicaltrials.gov/ct2/show/record/NCT00035620>.

<sup>46</sup> Neupogen® (filgrastim) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2012); and Neulasta® (pegfilgrastim) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2012).

<sup>47</sup> T. Hirama et al., “Initial Medical Management of Patients Severely Irradiated in the Tokai-mura Criticality Accident,” *Br J Radiol* 76 (2003): 264–53.

<sup>48</sup> IAEA, *The Radiological Accident in Gilan* (Vienna, Austria: IAEA, 2002).

<sup>49</sup> IAEA, *The Radiological Accident in Istanbul* (Vienna, Austria: IAEA, 2000).

<sup>50</sup> N. Dainiak et al., “First Global Consensus for Evidence-based Management of the Hematopoietic Syndrome Resulting from Exposure to Ionizing Radiation,” *Disaster Med Public Health Prep* 5 (2011): 202–12.

<sup>51</sup> IAEA, *The Radiological Accident in Yanango* (Vienna, Austria: IAEA, 2000).

<sup>52</sup> IAEA, *The Radiological Accident in Samut Prakarn* (Vienna, Austria: IAEA, 2002).

filgrastim in NHP model of ARS that included supportive care. In study AXR01,<sup>53</sup> the rhesus macaque model of HP ARS was characterized and described, including endpoints, supportive care treatments, and triggers to treat. In this extensive study, documentation of symptoms and cytopenia and cytopoiesis kinetics was made, as well as comparisons with known human values following IR exposure.

The second component of this endeavor, study AXG15,<sup>54</sup> compared the efficacy of filgrastim and supportive care to supportive care alone following IR. The pharmacokinetics (PK) and pharmacodynamics (PD) of filgrastim in NHP was described and used as a basis, along with human PK and PD data, to select the dose used. The primary endpoint of the study was the 60-day survival following an LD<sub>50/60</sub> dose, and 79% of filgrastim-treated animals survived versus 41% control. AXG15 implemented filgrastim subcutaneous injection every day beginning about 24 hours after IR until the absolute neutrophil count (ANC) was greater than or equal to  $1.0 \times 10^9 / L$  for three days (or  $10 \times 10^9 / L$  for two consecutive days up to study day 5 or any time after study day 5).

Several secondary endpoints were observed, as well. Regarding neutropenic parameters, while the mean nadir of neutropenia was not significantly affected by filgrastim administration, the time to neutrophil recovery was reduced in filgrastim-treated animals. Thrombocytopenic parameters were unchanged in response to filgrastim. Other endpoints showed no significant differences between treatment and control groups. The data from this study support the neutrophil recovery in the absence of other cellular level effects shown in Figure 5.

No DRF was calculated from the filgrastim NHP study. However, MacVittie, Farese, and Jackson performed a study in canines where they combined supportive care with G-CSF treatment. The DRF they calculated at the LD<sub>50/30</sub> was 1.88.<sup>55</sup>

#### **d. Alternate Dosing and Efficacy of Pegfilgrastim**

Pegfilgrastim is currently being studied in a similar model and the data are reported in Farese et al. However, the dosing schedule is one or two doses total—one on day 1 or one on day 1 and one on day 7 post-IR. Due to its longer elimination half-life, the total number of doses of pegfilgrastim is reduced compared to the daily dosing of filgrastim until ANC of greater than or equal to  $1.0 \times 10^9 / L$  is achieved, which is about 17–21 days. Pegfilgrastim levels become sub-therapeutic on day 6–7 if only the day 1 dose is

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<sup>53</sup> FDA, “Efficacy of Filgrastim in the Treatment of the Hematopoietic Syndrome of the Acute Radiation Syndrome Briefing Document,” 228.

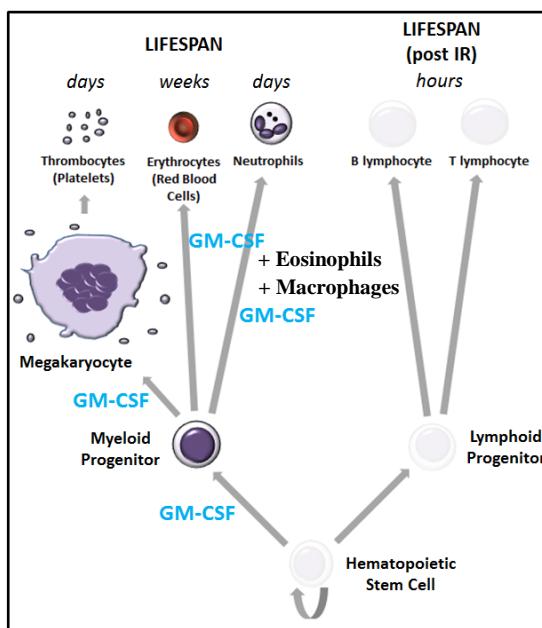
<sup>54</sup> Ibid; and A.M. Farese et al., “Filgrastim Improves Survival in Lethally Irradiated Nonhuman Primates,” *Radiat Res* 179 (2013): 89–100.

<sup>55</sup> MacVittie et al., “Defining the Full Therapeutic Potential of Recombinant Growth Factors in the Post Radiation-accident Environment,” 546–55.

given, and this is reflected in the greater neutrophil nadir in these animals compared to the nadirs of the two-dose regimen. The authors demonstrated that pegfilgrastim given twice at day 1 and day 7 is equal to or better than filgrastim given daily until neutrophil recovery.<sup>56</sup>

## 2. Sargramostim

Sargramostim is a synthetic form of human granulocyte-macrophage colony stimulating factor (GM-CSF) produced in *Saccharomyces cerevisiae* (*S. cerevisiae*). It is similar to filgrastim in its mechanism of action—stimulation of neutrophil recovery—and effect on blood cell recovery and survival post-IR. It is stored under refrigeration and can be injected subcutaneously. In addition to its role in generating new neutrophils, it also stimulates production of megakaryocytes, erythrocytes, eosinophils, and macrophages, as well as the parent cells of all of the above, which are the myeloid progenitor cells (see Figure 6).



**Figure 6. GM-CSF (Sargramostim) Stimulates Myelopoiesis, Leading to an Increase in Platelets, Erythrocytes, Neutrophils, Eosinophils, and Macrophages**

<sup>56</sup> A.M. Farese et al., “Pegfilgrastim Administered in an Abbreviated Schedule, Significantly Improved Neutrophil recovery after High-Dose Radiation-Induced Myelosuppression in Rhesus Macaques,” *Radiat Research* 178 (2012): 403–13.

### a. Human Safety of Sargramostim

Sargramostim is FDA-approved for use in neutrophil recovery from chemotherapy, mobilization of peripheral blood progenitor cells (PBPC), and myeloid reconstitution following bone marrow transplant.<sup>57</sup> Like filgrastim, sargramostim is recommended to be administered 24 to 72 hours following chemotherapy, though it can be given up to 120 hours following high-dose therapy.<sup>58</sup>

GM-CSF has been used off-label following radiation accidents. Dainiak et al. reviewed several cases where GM-CSF was used to treat HP ARS in patients from radiation accidents in Goiânia, Brazil (1987)<sup>59</sup> and Henan Province, China (1999).<sup>60</sup> Additional human experience with GM-CSF following IR exposure comes from accidents in San Salvador, El Salvador (1989);<sup>61</sup> Soreq, Israel (1990);<sup>62</sup> Nyasvizh, Belarus (1991);<sup>63</sup> and Samut Prakarn Province, Thailand (2000).<sup>64</sup>

### b. Efficacy of Sargramostim in Treating ARS

Sargramostim has been tested in numerous animal models of ARS in mouse, dog, and NHP, and it has a few disadvantages compared to its filgrastim cousins in ARS countermeasure research. The first is that sargramostim works at several points in hematopoiesis (Figure 6), whereas filgrastim preferentially enhances neutrophil proliferation (Figure 5). Since sargramostim targets cell proliferation other than neutrophils and primarily macrophage and eosinophil proliferation is enhanced, this choice of cell fate occurs at the expense of making more neutrophils.<sup>65</sup> While

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<sup>57</sup> Leukine® (sargramostim) [United States Prescribing Information] (Bridgewater, NJ: Sanofi-Aventis, 2012).

<sup>58</sup> Smith et al., “2006 Update of Recommendations for the Use of White Blood Cell Growth Factors, 3187–205.

<sup>59</sup> IAEA, *The Radiological Accident in Goiânia*.

<sup>60</sup> Q. Liu et al., “Clinical Report of Three Cases of Acute Radiation Sickness from a 60Co Radiation Accident in Henan Province in China,” *J Radiat Res* 49 (2008): 63–9; and N. Daniak et al., “First Global Consensus for Evidence-Based Management of the Hematopoietic Syndrome Resulting from Exposure to Ionizing Radiation,” 202–12.

<sup>61</sup> IAEA, *The Radiological Accident in San Salvador* (Vienna, Austria: IAEA, 1990).

<sup>62</sup> IAEA, *The Radiological Accident in Soreq* (Vienna, Austria: IAEA, 1993).

<sup>63</sup> IAEA, *The Radiological Accident at the Irradiation Facility In Nesvizh* (Vienna, Austria: IAEA, 1996).

<sup>64</sup> IAEA, *The Radiological Accident in Samut Prakarn*.

<sup>65</sup> M.I. Lord et al., “Myeloid Cell Kinetics in Mice Treated with Recombinant Interleukin-3, Granulocyte Colony-Stimulating Factor (CSF), or Granulocyte-Macrophage CSF in vivo,” *Blood* 77 (1991): 2154–9; and W.P. Hammond et al., “A Comparison of Treatment of Canine Cyclic Hematopoiesis with Recombinant Human Granulocyte-Macrophage Colony-Macrophage Stimulating Factor (GM-CSF), Interleukin-3, and Canine G-CSF,” *Blood* 76 (1990): 523–32.

macrophages may be important in fighting sepsis, which is the most common cause of death in ARS, eosinophils are unlikely to contribute to infection proliferation, since they function primarily in parasite infections and allergic responses. A second disadvantage is that human GM-CSF is not cross-reactive in mice, whereas human G-CSF can be used in mice, NHP, and humans. Since the FDA Animal Rule requires that a countermeasure be tested in at least two animal models, this makes it more difficult for sargramostim to be approved for ARS. A third potential disadvantage is that some research in chemotherapy-induced neutropenia demonstrated a higher incidence of fever in patients treated with GM-CSF.<sup>66</sup>

While sargramostim was more widely studied in animal models and prescribed following radiation accidents in humans in the earlier years of cytokine therapies, filgrastim is more prevalent in the lab, clinic, and following radiation accidents. While both sargramostim and filgrastim (and pegfilgrastim) are recommended for neutropenia in oncology settings,<sup>67</sup> some studies show that filgrastim is more effective with fewer side effects than sargramostim, while others show similar efficacy.<sup>68</sup> Furthermore, filgrastim is currently closer to being approved for use in ARS compared to sargramostim,<sup>69</sup> since it has already completed its pre-IND studies in NHP.<sup>70</sup> Notwithstanding filgrastim's preferential enhancement of neutrophil recovery over that of sargramostim, one would expect that on the basis of efficacy data, filgrastim is likely to be approved for ARS sooner than sargamostim.

## B. Potential Countermeasures That Are Indicated for Chemotherapy- or Radiotherapy-Induced Cytopenias and Could Be Repositioned to Treat ARS

Chemotherapy and radiotherapy to treat cancer can lead to the same cytopenias observed in ARS. While neutropenia is one of the most commonly-treated cytopenias

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<sup>66</sup> R.W. Dubois et al., “Benefits of GM-CSF versus Placebo in Reducing Chemotherapy-Induced Complications: A Systematic Review of the Literature,” *Support Cancer Ther* 2 (2004): 34–41.

<sup>67</sup> Smith et al., “2006 Update of Recommendations for the Use of White Blood Cell Growth Factors, 3187–205.

<sup>68</sup> For a short review comparing the efficacies of G-CSF and GM-CSF, see Smith et al. (above), p. 3196.

<sup>69</sup> Economic considerations that would lead to more visibility of filgrastim and pegfilgrastim (manufactured by Amgen) over sargramostim (manufactured by Genzyme) in the clinic and the literature, such as the marketing and company prioritization of the therapy were not addressed here. Pharmaceutical companies may prioritize certain therapies in their portfolios at various times, and these priorities may or may not reflect relative efficacies of the therapies. Whereas a potential contribution by company priorities to a therapy’s success cannot be discounted or affirmed, these factors are not addressed here.

<sup>70</sup> FDA, “Efficacy of Filgrastim in the Treatment of the Hematopoietic Syndrome of the Acute Radiation Syndrome Briefing Document,” 228.

during cancer treatment, anemia, bleeding, and GI disturbances also have FDA-approved treatments. It is possible that these could be repositioned as ARS therapies. Following are the advantages and further research required to reposition this class of therapies.

Advantages:

- These therapies have been shown to be effective against cytopenias, which would also be the symptom being treated in ARS.
- Their doses may be similar to those used for their current indications, so further safety data may not be necessary.
- Their use in similar indications provides further support of their potential efficacies in ARS.

Research required:

- Animal model efficacy studies would still need to be performed.
- There is no FDA-approved animal model to study ARS.

Below are some potential candidate therapies that could be repositioned from their current indications in treating side effects from cancer treatment. This is not an exhaustive list, but it includes some of the most commonly used methods beyond supportive care for treating the cytopenias that result from radiotherapy and chemotherapy. Inclusion in the list does not constitute a recommendation.

## 1. Palmifermin (Keratinocyte Growth Factor)

Palifermin is a synthetic human keratinocyte growth factor (KGF) produced in *E. coli*. It is FDA-indicated for oral mucositis following myelotoxic chemotherapy. It is stored under refrigeration and administered intravenously. Along with its clinically-approved indication for oral mucositis, animal studies have shown that it can protect the GI tract from IR when given prophylactically<sup>71</sup> (Figure 7), though the effect of its administration alone on overall survival is not currently known. (In conjunction with bone marrow transplant or methotrexate, KGF was shown to increase survival from ARS in mice.<sup>72</sup>) Hérodin and Drouet have suggested the KGF be considered as one of many cytokines to be used to treat high-dose IR.<sup>73</sup> However, in spite of its approval in the

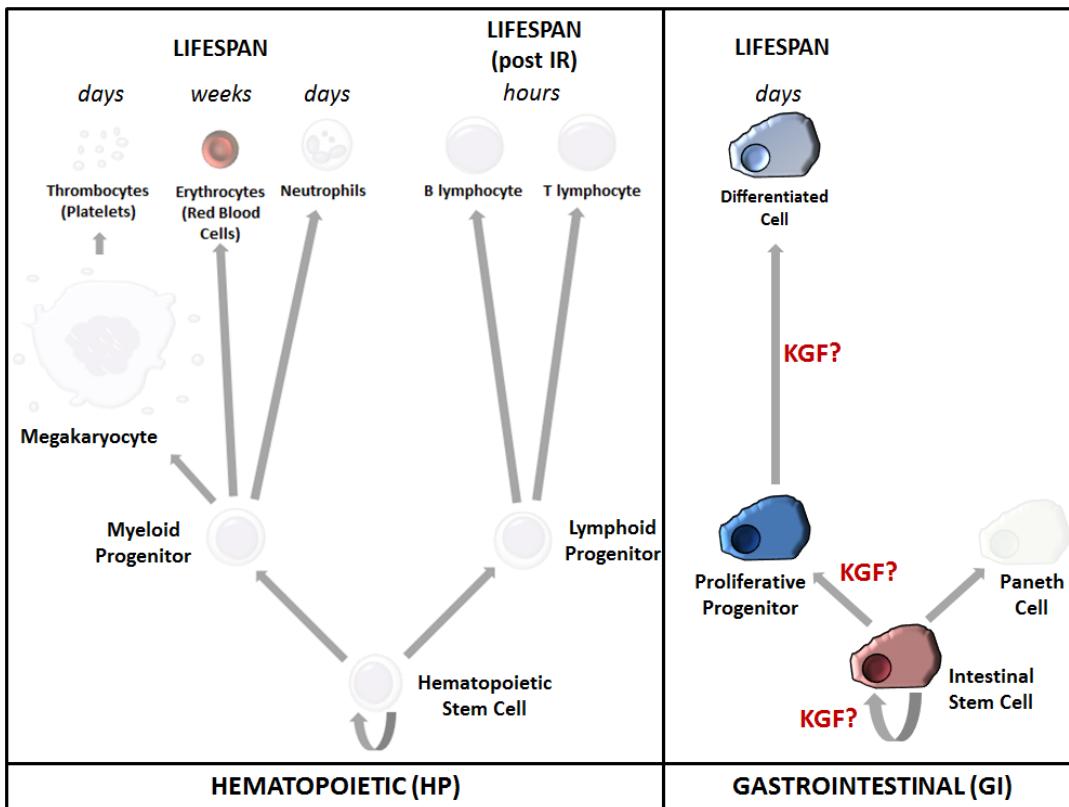
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<sup>71</sup> Y. Cai et al., “Keratinocyte Growth Factor Pretreatment Prevents Radiation-induced Intestinal Damage in a Mouse Model,” *Scand J Gastroenterol* 48 (2013): 419–26.

<sup>72</sup> C.L. Farrell et al., “Keratinocyte Growth Factor Protects Mice from Chemotherapy and Radiation-induced Gastrointestinal Injury and Mortality,” *Cancer Res* 58 (199):933–9.

<sup>73</sup> M. Drouet, and F. Hérodin, “Radiation Victim Management and the Haematologist in the Future: Time to Revisit Therapeutic Guidelines?” *Int J Radiat Biol* 86 (2010):636–48; and F. Hérodin F, and M. Drouet, “Cytokine-based Treatment of Accidentally Irradiated Victims and New Approaches,” *Experimental Hematology* 33 (2005):1071–80.

United States, its safety and efficacy profiles in treating chemotherapy-induced mucositis are still uncertain from some clinical trials,<sup>74</sup> and some suggest that palifermin requires more research<sup>75</sup> for ameliorating oral mucositis following chemotherapy or radiotherapy.



**Figure 7. KGF May Protect the GI Tract from IR-Induced Damage or Facilitate Recovery Following IR Exposure**

## 2. Interleukin-3 (IL-3)

Interleukin-3 (IL-3) was prescribed to patients following IR exposure in the Soreq radiation accidents in Israel (1990)<sup>76</sup> and Nyasvizh, Belarus (1991).<sup>77</sup> Animal studies

<sup>74</sup> M. Henke et al., “Palifermin Decreases Severe Oral Mucositis of Patients Undergoing Postoperative Radiochemotherapy for Head and Neck Cancer: A Randomized, Placebo-Controlled Trial,” *J Clin Oncol* 29 (2011): 2815–20; and Q.Y. Le et al., “Palifermin Reduces Severe Mucositis in Definitive Chemoradiotherapy of Locally Advanced Head and Neck Cancer: A Randomized, Placebo-Controlled Study,” *J Clin Oncol* 29 (2011): 2808–14.

<sup>75</sup> P. Bossi et al., “Palifermin in Prevention of Head and Neck Cancer Radiation-induced Mucositis: Not Yet a Definitive Word on Safety and Efficacy Profile,” *J Clin Oncol* 30 (2012): 564–5; and S.T. Sonis, “Efficacy of Palifermin (Keratinocyte Growth Factor-I) in the Amelioration of Oral Mucositis,” *Core Evidence* 4 (2009): 199–205.

<sup>76</sup> IAEA, *The Radiological Accident in Soreq*.

showed that recombinant IL-3 stimulated myelopoiesis.<sup>78,79</sup> though it is not currently being pursued clinically or pre-clinically for ARS or chemotherapy-induced neutropenia.

The chimeric IL-3 receptor and G-CSF receptor agonist, leridistim or myelopietin, showed initial promise in ameliorating neutropenia.<sup>80</sup> However, a Phase 3 trial that compared leridistim to G-CSF showed G-CSF to be superior in preventing febrile neutropenia following chemotherapy.<sup>81</sup>

### 3. Oprelvekin (Interleukin-11)

Oprelvekin is a synthetic form of human interleukin-11 (IL-11) produced in *E. coli* that stimulates thromopoiesis (Figure 8). It is indicated for preventing thrombocytopenia and reducing platelet transfusions following chemotherapy, though there is an increase in its toxicity following myeloablative therapy. Oprelvekin is stored under refrigeration and administered by subcutaneous injection.<sup>82</sup> Recombinant IL-11 has been used in animal studies of ARS<sup>83</sup> and shown to increase survival. There may be a role for IL-11 in IR-induced GI lesions, though more careful studies preventing death by HP ARS will need to be executed to validate intestinal changes with survival.<sup>84</sup>

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<sup>77</sup> IAEA, *The Radiological Accident at the Irradiation Facility In Nesvizh* (Vienna, Austria: IAEA, 1996).

<sup>78</sup> Lord et al., “Myeloid Cell Kinetics in Mice Treated with Recombinant Interleukin-3, Granulocyte Colony-Stimulating Factor (CSF), or Granulocyte-Macrophage CSF in vivo,” 2154–9.

<sup>79</sup> Hammond et al., “A Comparison of Treatment of Canine Cyclic Hematopoiesis with Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), G-CSF, Interleukin-3, and Canine G-CSF,” 523–32.

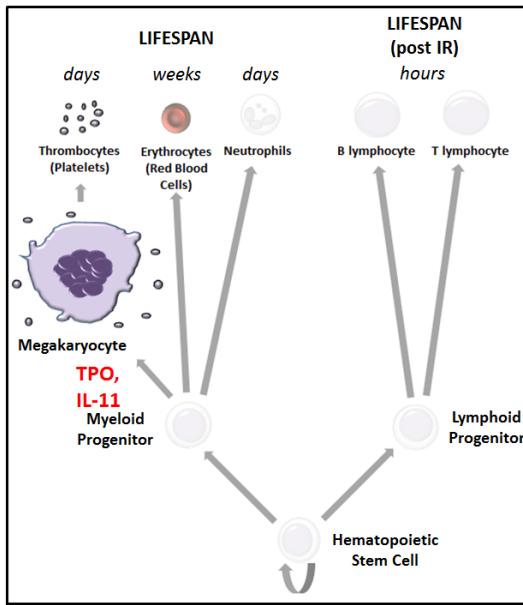
<sup>80</sup> T.J. MacVittie et al., “Myelopietin, An Engineered Chimeric IL-3 and G-CSF Receptor Agonist, Stimulates Multilineage Hematopoietic Recovery in a Nonhuman Primate Model of Radiation-induced Myelosuppression,” *Blood* 95 (2000): 837–45.

<sup>81</sup> Nabholz et al., “Phase III Trial Comparing Granulocyte Colony-Stimulating Factor to Leridistim in the Prevention of Neutropenic Complications in Breast Cancer Patients Treated with Docetaxel/Doxorubicin/Cyclophosphamide: Results of the BCIRG 004 Trial,” *Clin Breast Cancer* 3 (2002): 268–75.

<sup>82</sup> Neumega® (oprelvekin) [United States Prescribing Information] (Philadelphia, PA: Wyeth Pharmaceuticals Inc., 2011).

<sup>83</sup> A. Van der Meeren et al., “Administration of Recombinant Human IL11 after Supralethal Radiation Exposure Promotes Survival in Mice: Interactive Effect with Thrombopoietin,” *Radiat Res* 157 (2002):642–9.

<sup>84</sup> D. Booth, and C.S. Potten, “Protection against Mucosal Injury by Growth Factors and Cytokines,” *J Natl Cancer Inst Monogr* (2001): 16–20.



**Figure 8. TPO and IL-11 Preferentially Stimulate Platelet Production**

#### 4. Epoetin Alfa

Epoetin alfa is a synthetic human erythropoietin (EPO) produced in cell culture that stimulates erythropoiesis and is used to treat anemia. It is stored under refrigeration and injected subcutaneously. Its use in chemotherapy-induced anemia is controversial, and it is only indicated in cancers that do not have a high chance of being cured. Its use can cause serious and life-threatening reactions, including thromboembolic reactions. Furthermore, using it in ARS treatment may be limited due to the low rate of serious anemia in patients and the ability to temporarily replace erythrocytes with blood transfusions.<sup>85</sup> EPO was prescribed to patients following IR exposure in the Tokai-mura radiation incident in 1999<sup>86</sup> and in Henan Province, China in 1999,<sup>87</sup> though its efficacy in improving patient outcome is not known due to many confounding factors. For example, the patient in the Chinese incident who received EPO also received several packed red blood cell and platelet transfusions, as well as GM-CSF.<sup>88</sup>

<sup>85</sup> Epogen® (epoetin alfa) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2012); and Procrit® (epoetin alfa) [United States Prescribing Information] (Horsham, PA: Janssen Products, LP, 2000).

<sup>86</sup> H. Nagayama et al., “Transient Hematopoietic Stem Cell Rescue Using Umbilical Cord Blood for a Lethally Irradiated Nuclear Accident Victim,” *Bone Marrow Transplantation* 29 (2002): 197–204.

<sup>87</sup> Liu “Clinical Report of Three Cases of Acute Radiation Sickness from a 60Co Radiation Accident in Henan Province in China,” 63–9.

<sup>88</sup> Ibid.

## **C. Potential Countermeasures with Human Safety and Efficacy Data and FDA Approval for other Conditions That Could Be Repositioned to Treat ARS**

Therapies with indications that are unrelated to ARS might also prove effective in treating ARS. Below are advantages and further research required to reposition this class of therapies.

Advantages:

- Human safety data are available, and many of these therapies have been approved for a long time, yielding a history of human safety.
- Some efficacy data in treating ARS may be available.
- Many of these therapies act through mechanisms different from the more common cytokines that stimulate hematopoiesis, allowing for exploration into different mechanisms and targets for ARS treatments.

Research required:

- The doses used to treat their indications may be different from those required to treat ARS, so further safety studies will often be necessary.
- Efficacy data for treating ARS are usually limited, and animal model efficacy studies would still need to be performed.
- There is no FDA-approved animal model to study ARS.

Following are some potential candidate therapies that could be repositioned. This is not an exhaustive list, and inclusion in the list does not constitute a recommendation.

### **1. Romiplostim (Thrombopoietin)**

Romiplostim is a synthetic thrombopoietin (TPO) agonist that stimulates platelet production. Romiplostim is indicated for use in chronic immune (idiopathic) thrombocytopenia purpura (ITP).<sup>89</sup> It is stored under refrigeration and delivered by subcutaneous injection. It is currently in Phase 2 efficacy trials for chemotherapy-induced thrombocytopenia,<sup>90</sup> for which it is already prescribed off-label. It is indicated for use to obtain platelet counts greater than  $400 \times 10^9 / L$ , and it preferentially stimulates thrombopoiesis (Figure 8). TPO was administered to a patient of the Tokai-mura

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<sup>89</sup> Nplate® (romiplostim) [United States Prescribing Information] (Thousand Oaks, CA: Amgen Inc., 2008).

<sup>90</sup> ClinicalTrials.gov, “NCT01676961: Romiplostim in Increasing Low Platelet Counts in Patients with Multiple Myeloma Receiving Chemotherapy,” last updated October 11, 2013, <http://clinicaltrials.gov/show/NCT01676961>.

radiation incident, though the patient still succumbed to infection 210 days after IR exposure.<sup>91</sup>

Related to romiplostim is pegylated recombinant human megakaryocyte growth and development factor (peg-rHuMGDF), which also binds to the thrombopoietin receptor. peg-rHuMGDF was shown to enhance HP recovery in an NHP model of ARS, especially when combined with G-CSF.<sup>92</sup> However, while this drug showed promise in Phases 1 and 2 trials for thrombocytopenia following chemotherapy, a Phase 3 trial showed it to be no different than placebo in platelet recovery.<sup>93</sup>

## 2. Pasireotide

Pasireotide is a small molecule that mimics the natural peptide signaling molecule, somatostatin, stimulating four out of five somatostatin receptors with similar affinity to their activations by endogenous somatostatin. Pasireotide is currently FDA-approved for the treatment of Cushing's disease. Pasireotide is stored at room temperature and administered as a subcutaneous injection.<sup>94</sup>

It is currently being studied as a therapy for treating the GI syndrome following IR. Evidence suggests that pasireotide acts by preventing breakdown of the protective gut mucosa rather than by stimulating stem cell division or protecting from apoptosis,<sup>95</sup> which are the more common therapy mechanisms. Stimulating somatostatin receptors prevents the release of pancreatic enzymes, which can degrade the mucous in the gut, into the intestinal lumen. Maintaining the mucous lining of the gut can help prevent symptoms of GI syndrome after the permeabilization of the gut epithelia that eventually leads to sepsis. The protection of the gut by SOM230 is reversed by artificial administration of pancreatic enzymes,<sup>96</sup> supporting the current theory of its therapeutic mechanism.

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<sup>91</sup> Nagayama et al., "Transient Hematopoietic Stem Cell Rescue Using Umbilical Cord Blood for a Lethally Irradiated Nuclear Accident Victim," 197–204.

<sup>92</sup> A.M. Farese et al., "Combined Administration of Recombinant Human Megakaryocyte Growth and Development Factor and Granulocyte Colony-Stimulating Factor Enhances Multilineage Hematopoietic Reconstitution in Nonhuman Primates after Radiation-Induced Marrow Aplasia," *J Clin Investig* 97(1996): 2145–51.

<sup>93</sup> M.S. Schuster et al., "The Effects Of Pegylated Recombinant Human Megakaryocyte Growth And Development Factor (PEG-Rhumgdf) On Platelet Recovery In Breast Cancer Patients Undergoing Autologous Bone Marrow Transplantation," *Exp Hematol* 30 (2002): 1044–50.

<sup>94</sup> Signifor® (pasireotide) [United States Prescribing Information] (East Hanover, NJ: Novartis Pharmaceuticals Corporation, 2012).

<sup>95</sup> Q. Fu et al., "The Somatostatin Analog SOM230 (Pasireotide) Ameliorates Injury of the Intestinal Mucosa and Increases Survival after Total-Body Irradiation by Inhibiting Exocrine Pancreatic Secretion," *Radiat Res* 171 (2009): 698–707.

<sup>96</sup> Ibid.

ARS studies that focus on the GI often measure the LD<sub>50/10</sub>, which is the survival 10 days post-irradiation, a time point beyond crypt stem cell death and before HP syndrome. Pasireotide showed a reduction in GI syndrome in mice when administered up to 72 hours post-IR.<sup>97</sup> Other parameters measured showed that mucosal surface area was better preserved,<sup>98</sup> there was less bacterial translocation across the gut epithelia,<sup>99</sup> and there was a reduction in intestinal tissue proteolytic activity.<sup>100</sup>

Having already been FDA-approved for another indication, pasireotide could be repositioned with a new indication for ARS treatment, though doses reflecting its treatment of ARS would need to be addressed for safety. In 2011, the U.S. Department of Health and Human Services (DHHS) Biomedical Advanced Research and Development Authority (BARDA) awarded the University of Arkansas for Medical Sciences a contract to evaluate SOM230 in GI ARS.<sup>101</sup> However, it has currently only been studied in mice.

### 3. Phenylbutyrate

Phenylbutyrate is an inhibitor of histone deacetylase (HDAC). Phenylbutyrate is currently FDA-approved for the treatment of urea cycle disorders, and it can be administered orally and is stored at room temperature.<sup>102</sup>

Phenylbutyrate can protect leukocytes from radiation-induced apoptosis and increase neutrophils and platelets if administered 24 hours before IR.<sup>103</sup> Phenylbutyrate has already been shown to be safe in humans; however, it would now need to address the FDA Animal Rule to advance as an ARS therapy as well as safety given any differences in doses.

### 4. Beclomethasone Dipropionate (BDP)

BDP is a corticosteroid that has been used topically to reduce radiation-induced bleeding of the rectum in prostate cancer patients. It has been explored clinically for the

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<sup>97</sup> Q. Fu et al., “Preclinical Evaluation of SOM230 as a Radiation Mitigator in a Mouse Model: Postexposure Time Window and Mechanisms of Action,” *Radiat Res* 175 (2011): 728–35.

<sup>98</sup> Fu et al., “The Somatostatin Analog SOM230 (Pasireotide) Ameliorates Injury of the Intestinal Mucosa and Increases Survival after Total-body Irradiation by Inhibiting Exocrine Pancreatic secretion,” 698–707.

<sup>99</sup> Ibid.

<sup>100</sup> Fu et al. “Preclinical Evaluation of SOM230 as a Radiation Mitigator in a Mouse Model: Postexposure Time Window and Mechanisms of Action,” 728–35.

<sup>101</sup> DHHS, “BARDA Funds Development of Five Drugs to Protect against Radiation,” Sep 28, 2011.

<sup>102</sup> Buphenyl® (phenylbutyrate) [United States Prescribing Information] (South San Francisco, CA: Hyperion Therapeutics, Inc., 2009).

<sup>103</sup> A.C. Miller et al., “Radioprotection by the Histone Deacetylase Inhibitor Phenylbutyrate,” *Radiat Environ Biophys* 50 (2011): 585–96.

treatment of Crohn's disease<sup>104</sup> and gastrointestinal acute graft-versus-host disease (GvHD).<sup>105</sup> An inhalational formulation is also indicated for the treatment of asthma.<sup>106</sup> An oral formulation has recently gained orphan drug status, following pre-clinical results in a canine model of GI ARS.<sup>107</sup> The data, however, remain unpublished.

## 5. Ciprofloxacin

Ciprofloxacin is an FDA-approved fluoroquinolone antibiotic used to treat bacterial infections. In addition to its potential role in treating bacterial infections that can occur following IR exposure, ciprofloxacin is also being studied as a radiation therapy. It has primarily been studied in combined injury models, and has been shown to improve survival when mice are wounded and exposed to IR.<sup>108,109,110</sup>

## 6. Amifostine

Amifostine, also known as WR-2721, has been pursued as a radioprotectant for decades. It is a phosphothioate that acts as a free radical scavenger. Amifostine is administered intravenously (or intraperitoneally in mice) and can be stored at room temperature. It is FDA-approved for the prevention of radiation-induced xerostomia in head and neck cancer and to reduce renal toxicity of cisplatin treatment in ovarian cancer. When administered before IR, amifostine provides an impressive DRF at the LD<sub>50/30</sub> in mice of 2.25 for gamma-IR and a DRF of 1.41 for neutron IR.<sup>111</sup> However, due to its

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<sup>104</sup> M. Astegiano et al., "Efficacy and Safety of Oral Beclomethasone Dipropionate for Ileal or Ileal-Right Colon Crohn's Disease of Mild-to-Moderate Activity or In Remission: Retrospective Study," *Biomed Pharmacother* 61 (2007): 370–6.

<sup>105</sup> Y. Miura et al., "Oral Beclomethasone Dipropionate as an Initial Treatment of Gastrointestinal Acute Graft-Versus-Host Disease after Reduced-Intensity Cord Blood Transplantation," *Bone Marrow Transplantation* 38 (2006): 577–9.

<sup>106</sup> QVAR® (beclomethasone dipropionate HFA) [United States Prescribing Information] (Horsham, PA: Teva Respiratory, LLC, 2012).

<sup>107</sup> Soligenix, Inc, "FDA Grants Soligenix Orphan Drug Designation for OrbeShield™ for Treatment after Exposure to a Radiological Disaster," January 2, 2013.

<sup>108</sup> R. Fukumoto et al., "Ciprofloxacin Modulates Cytokine/Chemokine Profile in Serum, Improves Bone Marrow Repopulation, and Limits Apoptosis and Autophagy in Ileum after Whole Body Ionizing Irradiation Combined with Skin-Wound Trauma," *PLoS One* 8 (2013).

<sup>109</sup> R. Fukumoto, T. M. Burns, and J. G. Kiang, "Ciprofloxacin Enhances Stress Erythropoiesis in Spleen and Increases Survival after Whole-Body Irradiation Combined with Skin-Wound Trauma," *PLoS One* 9 (2014).

<sup>110</sup> J.G. Kiang, and R. Fukumoto, "Ciprofloxacin Increases Survival after Ionizing Irradiation Combined Injury: Gamma-H2AX Formation, Cytokine/Chemokine, and Red Blood Cells," *Health Physics* 10 (2014).

<sup>111</sup> J.S. Rasey et al., "Radioprotection of Normal Tissues against Gamma Rays and Cyclotron Neutrons with WR-2721: LD50 Studies and 35S-WR-2721 Biodistribution," *Radiat Res* 97 (1984): 598–607.

adverse side effect profile including severe vomiting and hypotension,<sup>112</sup> amifostine is not currently pursued as a radiation countermeasure.

#### **D. Non-FDA Approved Treatments with Human Safety and Non-Human Primate (NHP) Efficacy Data That Could Potentially Treat ARS: Examples of the Traditional Drug Development Model**

Several novel therapies that have no history of FDA approval for any indication are being developed either solely to treat ARS or also to treat ARS and for use in cancer treatments. Following are the advantages and further research required to reposition this class of therapies.<sup>113</sup>

Advantages:

- Many of these therapies have extensive data supporting their efficacy in treating ARS.
- Some of these therapies are also being researched as cancer treatments, providing market incentive for the company to pursue development of the therapy.
- Some therapies also have moved through Phase 1 safety trials.
- Many of these therapies act through mechanisms different from the more common cytokines that stimulate hematopoiesis, allowing for exploration into different mechanisms and targets for ARS treatments.
- In addition to the financial support of the companies developing the therapies, some of them are also currently also being supported by BARDA contracts or conducting research in conjunction with the Armed Forces Radiobiology Research Institute (AFRRI).

Research required:

- Some therapies still require human safety data.
- It appears that further efficacy data for treating ARS are required in all of these therapies to satisfy the FDA Animal Rule. There is no FDA-approved animal model to study ARS.

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<sup>112</sup> Ethyol® (amifostine) [United States Prescribing Information] (Nijmegen, The Netherlands: MedImmune, 2013).

<sup>113</sup> Since these therapies are all being developed by private companies, IDA's knowledge is limited to data available in the open literature. IDA attempted to obtain proprietary data from the companies, but the companies did not provide any.

Following are some potential candidate therapies that are being developed via traditional drug development routes. This is not an exhaustive list, and inclusion in the list does not constitute a recommendation.

## 1. Interleukin-12 (IL-12)

Interleukin-12 (IL-12) is a pro-inflammatory cytokine. Recombinant human IL-12 (rHuIL-12) is being studied as a radiation therapy and can be administered subcutaneously. rHuIL-12 has both human safety and NHP efficacy data. Endogenous IL-12 affects many parts of the immune system, especially upregulating the cytotoxic effects of T-lymphocytes and natural killer cells. The exact mechanism of the therapeutic effects of rHuIL-12 following IR are not completely identified; however, Basile et al. showed the prevalence of IL-12 receptors in bone marrow and intestinal crypts from non-irradiated humans and NHP.<sup>114</sup>

### a. Human Safety of rHuIL-12

In 2012, Phase 1 of the rHuIL-12 safety trial in humans was completed,<sup>115</sup> and the data from this clinical trial have recently been published, showing that rHuIL-12 is safe up to a single dose of 12 micrograms ( $\mu\text{g}$ ).<sup>116</sup>

Some concern has been raised about the safety of recombinant human IL-12. IL-12 was pursued for its anti-tumor properties in the 1990s, and the initial Phase 1 clinical trials in healthy humans showed safety.<sup>117</sup> However, a Phase 2 clinical trial administering a dose of 500 nanograms (ng)/kilogram (kg) intravenously in patients with advanced renal cell carcinomas led to a series of adverse events culminating in 12 hospitalizations and 2 deaths.<sup>118</sup> The dose used in the 1990s clinical trial is the equivalent of 35  $\mu\text{g}$  for a 70 kg human.<sup>119</sup> The NHP doses used in the current rHuIL-12 efficacy studies for ARS in NHPs are between 50 and 500 ng/kg for survival studies following IR,<sup>120</sup> including a

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<sup>114</sup> L.A. Basile et al., “HemaMax, A Recombinant Human Interleukin-12, Is a Potent Mitigator of Acute Radiation Injury in Mice and Non-Human Primates,” *PLOS One* 7 (2012): 1–23.

<sup>115</sup> ClinicalTrials.gov, “NCT01742221: Safety and Tolerability of HemaMaxTM (rHuIL-12) as Radiation Countermeasure,” last updated April 25, 2013, <http://clinicaltrials.gov/ct2/show/record/NCT01742221>.

<sup>116</sup> M.S. Gokhale et al., “Single Low-dose rHuIL-12 Safely Triggers Multilineage Hematopoietic and Immune-mediated Effects,” *Experimental Hematology and Oncology* 3 (2014).

<sup>117</sup> J.P. Leonard et al., “Effects of Single-dose Interleukin-12 Exposure on Interleukin-12 Associated Toxicity and Interferon-gamma Production,” *Blood* 90 (1997): 2541–8.

<sup>118</sup> Ibid.

<sup>119</sup> Ibid.

<sup>120</sup> Basile et al., “HemaMax™, A Recombinant Human Interleukin-12, Is a Potent Mitigator of Acute Radiation Injury in Mice and Non-Human Primates,” 1–23; and Gluzman-Poltorak et al., “Randomized Comparison of Single Dose of Recombinant Human IL-12 Versus Placebo for Restoration of Hematopoiesis And Improved Survival in Rhesus Monkeys Exposed to Lethal Radiation.”

more extensively studied dose of 175 ng/kg, which is the equivalent to a 12 µg dose for a 70 kg human.<sup>121</sup>

The dose used in the current studies with rHuIL-12 and tolerated in Phase 1 safety trials is about half the dose of the similar compound that caused illness in the 1990s, which is not a marked difference. It is unclear whether IL-12 in its current formulation would see similar adverse events. However, in the Phase 1 efficacy trials, doses of rHuIL-12 up to 20 µg were administered to healthy humans. This dose was scaled back to 15 µg and then to 12 µg, and the authors cite toxicity as the primary reason for the 12 µg dose.<sup>122</sup>

### b. Efficacy of rHuIL-12 in Treating ARS

The animal studies with administration of rHuIL-12, 24 to 25 hours after TBI in NHPs, are promising, with improved survival in both when either an LD<sub>90/60</sub> or LD<sub>50/30</sub> dose was administered. Furthermore, animals in these studies had more neutrophils, lymphocytes, thrombocytes, and erythrocytes at nadir.<sup>123</sup> rhHuIL-12 has also been studied in tumor-bearing mice with chemotherapy- or radiotherapy-induced myelosuppression, where the efficacy of rHuIL-12 in improving blood cell counts was compared to the efficacy of G-CSF, and the rHuIL-12-treated mice had similar or better blood cell count recoveries than did those receiving G-CSF.<sup>124</sup> Furthermore, rHuIL-12 was compared to G-CSF in a recent study, as well, and rHuIL-12 at 175 ng/kg improved survival, and G-CSF was shown to be no more effective than vehicle at treating ARS. However, these studies were done in the absence of any supportive care to the NHPs, and further research will be required to determine if the lack of supportive care explains the lack of efficacy of G-CSF in treating ARS.<sup>125</sup>

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<sup>121</sup> Z. Gluzman-Poltorak, V. Vainstein, and L. A. Basile, “Recombinant Interleukin-12, but Not Granulocyte-Colony Stimulating Factor, Improves Survival In Lethally Irradiated Non-Human Primates in the Absence of Supportive Care: Evidence for the Development of a Frontline Radiation Medical Countermeasure,” *American Journal of Hematology* 89, no. 9 (2014): 868–873.

<sup>122</sup> Gokhale et al., “Single Low-dose rHuIL-12 Safely Triggers Multilineage Hematopoietic and Immune-mediated Effects.”

<sup>123</sup> Basile et al., “HemaMax™, A Recombinant Human Interleukin-12, Is a Potent Mitigator of Acute Radiation Injury in Mice and Non-Human Primates,” 1–23; and Gluzman-Poltorak et al., “Randomized Comparison of Single Dose of Recombinant Human IL-12 Versus Placebo for Restoration of Hematopoiesis and Improved Survival in Rhesus Monkeys Exposed to Lethal Radiation.”

<sup>124</sup> L.A. Basile et al., “Multilineage Hematopoietic Recovery with Concomitant Antitumor Effects Using Low Dose Interleukin-12 in Myelosuppressed Tumor-Bearing Mice,” *J Transl Med* 6 (2008): 26.

<sup>125</sup> Gluzman-Poltorak et al., “Recombinant Interleukin-12, but Not Granulocyte-Colony Stimulating Factor, Improves Survival in Lethally Irradiated Non-Human Primates in the Absence of Supportive Care: Evidence for the Development of a Frontline Radiation Medical Countermeasure,” 868–873.

## **2. Entolimod**

Entolimod, also known as CBLB502, is a biologic therapy mimicking the *Salmonella* protein, flagellin, and is expressed in *E. coli*. It can be delivered by subcutaneous or intramuscular injection. Entolimod can stimulate toll-like receptor 5 (TLR5) leading to nuclear factor-kappa B (NF $\kappa$ B) activation.

### **a. Human Safety of Entolimod**

It does not appear that entolimod is being pursued with an IND as a radiation countermeasure at this time. However, entolimod also has a potential application in halting tumor cell growth and suppressing the growth of liver metastases.<sup>126</sup> It is currently in a Phase 1 clinical trial for patients with solid tumors.<sup>127</sup>

### **b. Efficacy of Entolimod in Treating ARS**

In both murine and primate models, CBLB502 is effective in increasing survival and improving the HP and GI systems,<sup>128</sup> as well as protecting mice from oral mucositis in a local radiation model.<sup>129</sup> The effect on HP and GI systems as well as survival following IR is mediated, at least in part, through its upregulation of endogenous G-CSF and interleukin-6.<sup>130</sup>

## **3. 5-Androstenediol (5-AED)**

5-AED is a naturally-produced steroid that can be administered subcutaneously or intramuscularly. Although DHHS originally supported the development of 5-AED for potential inclusion in the U.S. Project BioShield program and received IND status in 2005, DHHS is no longer pursuing its development.<sup>131</sup>

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<sup>126</sup> L.G. Burdelya et al., “Central Role of Liver in Anticancer and Radioprotective Activities of Toll-Like Receptor 5 Agonist,” *PNAS* 110 (2013): E1857–66.

<sup>127</sup> ClinicalTrials.gov, “NCT01527136: Entolimod in Treating Patients with Locally Advanced or Metastatic Solid Tumors that Cannot Be Removed by Surgery,” last updated January 10, 2014, <http://clinicaltrials.gov/show/NCT01527136>.

<sup>128</sup> L.G. Burdelya et al., “An Agonist of Toll-like Receptor 5 has Radioprotective Activity in Mouse and Primate Models,” *Science* 320 (2008): 226–30.

<sup>129</sup> L.G. Burdelya et al., “Toll-like Receptor 5 Agonist Protects Mice from Dermatitis and Oral Mucositis Caused by Local Radiation: Implications for Head-and-Neck Cancer Radiotherapy,” *Int J Radiat Oncol Biol Phys* 83 (2012): 228–34.

<sup>130</sup> V.I. Krivokrysenko et al., “Identification of Granulocyte Colony-Stimulating Factor and Interleukin-6 as Candidate Biomarkers of CBLB502 Efficacy as a Medical Radiation Countermeasure,” *J Pharmacol Exp Ther* 343 (2012): 497–508.

<sup>131</sup> J. R. Raj, “UPDATE 2-Hollis-Eden to Curtail Devt of Neumune, Shares Rise,” Reuters, Mar 16, 2007.

### **a. Human Safety of 5-AED**

5-AED completed successful Phase 1 clinical trials for the safety of 5-AED.<sup>132</sup>

### **b. Efficacy of 5-AED**

5-AED has been shown in mice and NHPs to be effective as a therapy for ARS. Mice treated with 5-AED before or after IR exposure showed enhanced survival. While 5-AED does not affect loss of lymphocytes or erythrocytes post-IR, 5-AED treatment reduces neutropenia and thrombocytopenia and enhances resistance to infection with *Klebsiella pneumonia*. 5-AED administration in unirradiated mice also stimulates neutrophil and platelet production and circulation.<sup>133</sup> Improved survival following IR as a result of 5-AED administration is mediated by elevated G-CSF expression, as well as preventing apoptosis.<sup>134</sup> In an NHP model without supportive care, 5-AED administration after IR improved survival and shortened the duration of thrombocytopenia and neutropenia.<sup>135</sup> There has also been further research on subcutaneous injection of micro- and nanoparticle formulations of 5-AED for ARS in NHP.<sup>136</sup>

## **4. Recilisib**

Recilisib is a synthetic chlorobenzylsulfone derivative. It was originally named ON01210.Na and can be delivered subcutaneously<sup>137</sup> or orally.<sup>138</sup>

### **a. Human Safety of Recilisib**

Recilisib is currently in IND status with the FDA. Three Phase 1 safety trials in humans were completed for recilisib, studying safety and the pharmacokinetics of oral

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<sup>132</sup> Ibid.

<sup>133</sup> M.H. Whitnall et al., “Androstenediol Stimulates Myelopoiesis and Enhances Resistance to Infection in Gamma-irradiated Mice,” *Int J Immunopharm* 22 (2000): 1–14.

<sup>134</sup> M.B. Grace et al., “5-AED Enhances Survival of Irradiated Mice in a G-CSF-dependent Manner, Stimulates Innate Immune Cell Function, Reduces Radiation-induced DNA Damage and Induces genes that Modulate Cell Cycle Progression and Apoptosis,” *J Radiat Res* 53 (2012): 840–53.

<sup>135</sup> D.R. Stickney et al., “5-androstenediol Improves Survival in Clinically Unsupported Rhesus Monkeys with Radiation-induced Myelosuppression,” *Int Immunopharm* 7 (2007): 500–5.

<sup>136</sup> Ibid.

<sup>137</sup> A.W. Chun et al., “Effects of Formulation and Route of Administration on the Systemic Availability of EX-RAD®, A New Radioprotectant, in Preclinical Species,” *Biopharm Drug Dispos* 32 (2011): 99–111.

<sup>138</sup> S. Suman et al., “Radioprotective Effects of ON 01210.Na upon Oral Administration,” *J Radiat Res* 53 (2012):368–76.

and subcutaneous administration.<sup>139</sup> However, the results of this study are not yet publicly available for review.

### **b. Efficacy of Recilisib in Treating ARS**

All efficacy data to date is in rodent models. Recilisib will likely need to be studied in an NHP model to satisfy demonstration of efficacy. However, in mice recilisib administration before exposure protects from both the HP and GI ARS syndromes.<sup>140</sup> Several studies<sup>141</sup> support the role of recilisib in preventing apoptosis and activating pro-survival pathways in the bone marrow, GI tract, and spleen.

Recilisib can also be effective in protecting the HP system when administered post-IR. There is evidence that upregulation of anti-apoptotic and pro-survival pathways also mediate this effect.<sup>142</sup>

## **E. Potential Countermeasures in Early Clinical, Pre-Investigational New Drug (IND) Application Candidate Screening, or Basic Research Strategies**

The following are some examples of countermeasures in early clinical, pre-IND, drug candidate screening, or basic research stages. There are many more potential countermeasures in this category, and nothing is implied by their exclusions or inclusions.

### **1. ALXN4100TPO**

ALXN4100TPO is a TPO receptor agonist. In mouse models of ARS, it shows efficacy in preventing death from ARS when administered both before and after IR.<sup>143</sup> However, it has no human safety or NHP efficacy data.

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<sup>139</sup> Onconova, “Onconova Presents Positive Clinical Trial results for Radiation Injury Protector Ex-RAD®,” Oct 3, 2012.

<sup>140</sup> S.P. Ghosh et al., “Amelioration of Radiation-induced Hematopoietic and Gastrointestinal Damage by Ex-RAD® in Mice,” *J Radiat Res* 53 (2012): 526–36.

<sup>141</sup> Ibid; S. Suman et al., “Administration of ON 01210.Na after Exposure to Ionizing Radiation Protects Bone Marrow Cells by Attenuating DNA Damage Response,” *Radiat Oncol* 7 (2012): 6; and A.D. Kang et al., “ON01210.Na (Ex-RAD®) Mitigates Radiation Damage through Activation of the AKT Pathway,” *PLOS One* 8, no.3 (2013): e58355.

<sup>142</sup> Suman et al., “Administration of ON 01210.Na after Exposure to Ionizing Radiation Protects Bone Marrow Cells by Attenuating DNA Damage Response,” 6.

<sup>143</sup> M. Satyamitra et al., “A TPO Receptor Agonist, ALXN4100TPO, Mitigates Radiation-induced Lethality and Stimulates Hematopoiesis in CD2F1 Mice,” *Radiat Res* 175 (2011): 746–58.

## **2. CBLB612 and CBLB613**

Cleveland Biolabs is currently pursuing research on other toll-like receptor agonists other than entolimod. CBLB612<sup>144</sup> and CBLB613<sup>145</sup> act on the Toll-like receptor 2 (TLR2) and Toll-like receptor 6 (TLR6), respectively. They are synthetic or naturally occurring lipopeptides and stimulate hematopoiesis.

## **3. Genistein**

Genistein is a soy-derived isoflavone. It is sold as a health supplement, and it is associated with ameliorating cardiovascular disease, high cholesterol, breast and prostate cancer, and osteoporosis. Genistein is currently in five Phase 1 and 2 clinical trials, usually in combination with other therapies, for the treatment of various cancers.<sup>146</sup> It has no IND status, however, for treating ARS.

Subcutaneous administration of genistein prior to IR improves survival in mice.<sup>147</sup> This may be partly mediated through genistein-mediated induction of G-CSF and IL-6 in both irradiated and non-irradiated mice.<sup>148</sup> There is also evidence that genistein is protective of HP stem cells by inducing their quiescence.<sup>149</sup>

## **4. Vitamin E Derivatives**

Vitamin E includes tocopherols and tocotrieneols of various isoforms. Most studies of vitamin E derivatives have been performed with alpha-tocopherol, though two other isoforms, delta-tocotrienol and gamma-tocotrienol, show more efficacy for

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<sup>144</sup> A.N. Shakhov et al., “Prevention and Mitigation of Acute Radiation Syndrome in Mice by Synthetic Lipopeptide Agonists of Toll-Like Receptor 2 (TLR2),” *PLOS One* 7 (2012): 1–12.

<sup>145</sup> V.K. Singh et al., “CBLB613: A TLR2/6 Agonist, Natural Lipopeptide of Mycoplasma Arginini, as a Novel Radiation Countermeasure,” *Radiat Res* 177 (2012): 628–42.

<sup>146</sup> ClinicalTrials.gov, “NCT01628471: MTD Determination, Safety and Efficacy of the Decitabine-Genistein Drug Combination in Advanced Solid Tumors and Non-Small Cell Lung Cancer,” last updated January 17, 2013, <http://clinicaltrials.gov/show/NCT01628471>; ClinicalTrials.gov, “NCT01126879: Genistein in Treating Patients with Prostate Cancer,” last updated September 17, 2013, <http://clinicaltrials.gov/show/NCT01126879>; ClinicalTrials.gov, “NCT01325311: Cholecalciferol and Genistein before Surgery in Treating Patients with Early Stage Prostate Cancer,” last updated October 7, 2013, <http://clinicaltrials.gov/show/NCT01325311>; ClinicalTrials.gov, “NCT01489813: Study of Genistein in Reducing Side Effects of Superficial Bladder Cancer Treatment,” last updated July 30, 2013, <http://clinicaltrials.gov/show/NCT01489813>; and ClinicalTrials.gov, “NCT01538316: Prostate Cancer Prevention Trial with Quercetin and Genistein (QUERGEN),” last updated May 13, 2013, <http://clinicaltrials.gov/show/NCT01538316>.

<sup>147</sup> M. R. Landauer, V. Srinivasan, and T. M. Seed, “Genistein Treatment Protects Mice from Ionizing Radiation Injury,” *J Appl Toxicol* 23 (2003): 379–85.

<sup>148</sup> V.K. Singh et al., “Effects of Genistein Administration on Cytokine Induction in Whole-Body Gamma Irradiated Mice,” *Int Immunopharmac* 9 (2009): 1401–10.

<sup>149</sup> T.A. Davis et al., “Genistein Induces Radioprotection by Hematopoietic Stem Cell Quiescence,” *Int J Radiat Biol* 84 (2008): 713–26.

radioprotection.<sup>150</sup> Of comparable efficacy is tocopherol succinate. All vitamin E derivatives can all be injected subcutaneously, and they show a range of DRFs in mice.<sup>151</sup>

Vitamin E derivatives are well-known antioxidants, and some of their radioprotective effects are likely due to free radical scavenging. However, there is evidence that they can inhibit apoptosis and stimulate cell proliferation.<sup>152</sup> Tocopherol succinate protects against both the HP<sup>153</sup> and GI<sup>154</sup> syndromes, and this effect is at least partly mediated by G-CSF.<sup>155</sup> Vitamin E derivatives are not yet in clinical trials for the treatment of ARS.

## 5. ARA 290

ARA 290 is an immune system modulator and was granted a BARDA award in 2011 for further study of ARA 290 in ARS animal models.<sup>156</sup> In mouse models of HP and GI syndromes, subcutaneous administration of ARA 290 post-IR increased survival following lethal radiation.<sup>157</sup> It has no human safety or NHP efficacy data.

## 6. Rx100

Rx100 is a small molecule that inhibits apoptosis and enhances cell survival when administered subcutaneously 48 to 72 hours after IR.<sup>158</sup> Rx100 was granted a BARDA

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<sup>150</sup> For a review of Vitamin E derivatives, see V. K. Singh, L A. Beattie, and T. M. Seed, “Vitamin E: Tocopherols and Tocotrienols as Potential Radiation Countermeasures,” *J Radiat Res* (2013): 1–16.

<sup>151</sup> V.K. Singh et al., “Tocopherol Succinate: Modulation of Antioxidant Enzymes and Oncogene Expression, and Hematopoietic Recovery,” *Int J Radiat Oncol Biol Phys* 79 (2011): 571–8; V.K. Singh et al., “Radioprotective Properties of Tocopherol Succinate against Ionizing Radiation in Mice,” *J Radiat Res* 54 (2013): 210–20; and P.K. Singh et al., “Alpha-tocopherol Succinate Protects Mice against Radiation-induced Gastrointestinal Injury,” *J Radiat Res* 177 (2012): 133–45.

<sup>152</sup> Singh et al., “Radioprotective Properties of Tocopherol Succinate against Ionizing Radiation in Mice,” 210–20.

<sup>153</sup> Singh et al., “Tocopherol Succinate: Modulation of Antioxidant Enzymes and Oncogene Expression, and Hematopoietic Recovery,” 571–8.

<sup>154</sup> Singh et al., “Alpha-tocopherol Succinate Protects Mice against Radiation-induced Gastrointestinal Injury,” 133–45.

<sup>155</sup> P.K. Singh et al., “Radioprotective Efficacy of Tocopherol Succinate is Mediated through Granulocyte-Colony Stimulating Factor,” *Cytokine* 56 (2011): 411–21.

<sup>156</sup> DHHS, “BARDA Funds Development of Five Drugs to Protect against Radiation.”

<sup>157</sup> C. Orschell et al., “ARA 290 is an Efficacious Radiomitigator of Both the Hematopoietic and Gastrointestinal Syndromes of the Acute Radiation Syndrome,” Abstracts of the 55th Annual Meeting of the Radiation Research Society Savannah, October 4–7, 2009, 143.

<sup>158</sup> RxBio, Inc, “Rx100 Rescues Life If Administered Up to 72 Hours after Exposure to Lethal, Whole-body Radiation!” September 14, 2009.

award in 2011 for further study of Rx100 in GI ARS animal models as well as developing GMP for the drug.<sup>159</sup> It does not yet have human safety or NHP efficacy data.

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<sup>159</sup> DHHS, “BARDA Funds Development of Five Drugs to Protect against Radiation.”



## 4. Assessing Countermeasure Efficacy: Nuclear Weapons Detonation Illustrative Example

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Ultimately, a radiation medical countermeasure with maximal efficacy is desired. Currently, however, there exist supportive care and, possibly, leukocyte growth factors. Both provide protection of less than two LD<sub>50</sub>s, or a DRF of 1.5 for supportive care alone and 1.88 for filgrastim in addition to supportive care. These treatments target the HP subsyndrome of ARS, but neither has much effect on the GI subsyndrome, since the IR doses that they allow a person to survive are just at the border of GI ARS.

The primary objective of all radiation medical countermeasure development is to reduce the number of fatalities among those suffering from ARS in the aftermath of a radiation incident. The *AMedP-8(C)* CBRN casualty estimation methodology can be applied both to describe the extent to which this objective can be met with current treatment and to determine the DRF necessary to provide maximal efficacy under the second research strategy.

The details of how the *AMedP-8(C)* methodology is implemented for these purposes can be found in Appendix C. Here, two different scenarios involving the detonation of a nuclear weapon or improvised nuclear device are presented.<sup>160</sup> The first scenario is a detonation in a civilian population center, in this case Washington, DC, and the second is a detonation targeting a military population, in this case a heavy brigade combat team in the offensive (HBCT-O). Both scenarios consider various nuclear yields and heights of burst. Fatalities are estimated by superimposing blast, burn, and radiation radii from ground zero over a static population distribution.

In the following tables, estimated fatalities fall into two categories: (1) fatalities from prompt radiation alone (“RAD”) and (2) fatalities from all effects—blast, burn, and prompt radiation (“TOTAL”).<sup>161</sup> The former is a subset of the latter.

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<sup>160</sup> Radiological dispersal devices and radiological exposure devices scenarios are not included since they are not estimated to result in a significant number of fatalities from IR.

<sup>161</sup> Although the *AMedP-8(C)* methodology can consider the contribution of fallout to estimated fatalities, it does not do so here as these scenarios and estimated fatalities are largely notional and the added complexity of fallout calculations would not add to discussion.

Table 1 and Table 2 show the estimated fatalities in the Washington, DC and heavy brigade scenarios, respectively, both in the absence of any treatment whatsoever (a worst case) and with supportive care, which is assumed to have a DRF of 1.5. Details on how the DRF is used to change fatality estimates can be found in Appendix C.

**Table 1. Estimated Fatalities without Treatment and with Supportive Care in an Urban Population—Washington, DC**

		Ground Release			Low Air Release		
		1 kT	10 kT	50 kT	1 kT	10kT	50 kT
<b>No treatment</b>	RAD	11,700	15,900		12,000	5,380	
	<b>TOTAL</b>	12,700	28,600	47,900	13,100	24,500	82,700
<b>Supportive care</b>	RAD	9,790	14,100		9,700	2,880	
	(%Δ)	(-16%)	(-11%)		(-19%)	(-46%)	
<b>TOTAL</b>	10,800	26,800	47,900	10,800	22,000	82,700	
	(%Δ)	(-15%)	(-6.3%)	(0%)	(-18%)	(-10%)	(0%)

Note 1: %Δ is the percent change in either radiation only or total fatalities as a result of treatment.

Note 2: The DRF for supportive care is 1.5.

**Table 2. Estimated Fatalities without Treatment and with Supportive Care in an Military Deployment Scenario—Heavy Brigade Combat Team in the Offensive**

		Ground Release			Low Air Release		
		1 kT	10 kT	50 kT	1 kT	10kT	50 kT
<b>No treatment</b>	RAD	83	94		77	34	
	<b>TOTAL</b>	89	137	240	95	129	422
<b>Supportive care</b>	RAD	72	78		67	16	
	(%Δ)	(-13%)	(-17%)		(-13%)	(-53%)	
<b>TOTAL</b>	77	121	240	85	111	422	
	(%Δ)	(-13%)	(-12%)	(0%)	(-11%)	(-14%)	(0%)

Note 1: %Δ is the percent change in either radiation only or total fatalities as a result of treatment.

Note 2: The DRF for supportive care is 1.5.

The same methodology can be used to estimate the reduction in fatalities that would result if the population can be treated with filgrastim and supportive care. To illustrate the impact of the use of these therapies, Table 3 and Table 4 show the reduction in fatalities in an urban population and a military deployment scenario, respectively.<sup>162</sup>

<sup>162</sup> Filgrastim is used as an example because a DRF of its administration in conjunction with supportive care has been estimated.

**Table 3. Estimated Fatalities without Treatment and with Filgrastim and Supportive Care in an Urban Population—Washington, DC**

	RAD	Ground Release			Low Air Release		
		1 kT	10 kT	50 kT	1 kT	10kT	50 kT
<b>Filgrastim/ Supportive care</b>	<b>RAD</b>	9,050	1,1400		9,000	1,600	
	(%Δ)	(-23%)	(-28%)		(-25%)	(-70%)	
	<b>TOTAL</b>	10,100	24,000	47,900	10,100	20,800	82,700
	(%Δ)	(-20%)	(-16%)	(0%)	(-23%)	(-15%)	(0%)

Note 1: %Δ is the percent change in either radiation only or total fatalities as a result of treatment.

Note 2: The DRF for supportive care and filgrastim is 1.88.

**Table 4. Estimated Fatalities without Treatment and with Filgrastim and Supportive Care in an Military Deployment Scenario—Heavy Brigade Combat Team in the Offensive**

	RAD	Ground Release			Low Air Release		
		1 kT	10 kT	50 kT	1 kT	10kT	50 kT
<b>Filgrastim/ Supportive care</b>	<b>RAD</b>	65	69		61	6	
	(%Δ)	(-22%)	(-27%)		(-21%)	(-82%)	
	<b>TOTAL</b>	71	112	240	79	101	422
	(%Δ)	(-20%)	(-18%)	(0%)	(-17%)	(-22%)	(0%)

Note 1: %Δ is the percent change in either radiation only or total fatalities as a result of treatment.

Note 2: The DRF for supportive care and filgrastim is 1.88.

To determine the “limiting DRF” postulated in the second research strategy—the DRF beyond which no additional radiation fatalities could be saved in a nuclear detonation—the same methodology and scenarios were used. Theoretically, a radiation medical countermeasure with this DRF would have maximal efficacy; fatalities would be minimized and any countermeasure with a larger DRF would not have any marginal benefit. The limiting DRF for various nuclear yields and heights of burst can be found in Table 5. For the 50 kT yields, the concept is not applicable because blast effects dominate and there are no radiation-only fatalities. It is important to note that these DRFs are population distribution independent. Further details of how the limiting DRF was calculated can be found in Appendix C.

**Table 5. Limiting DRFs**

	Ground Release			Low Air Release		
	1 kT	10 kT	50 kT	1 kT	10kT	50 kT
<b>Limiting DRF</b>	161	18	N/A	52	2	N/A

To summarize the fatality estimates provided in the previous tables, Table 6 shows the percentage of total nuclear incident fatalities—those from blast, thermal, and radiation—that could be avoided with successful fielding of a medical radiation countermeasure as a consequence of either of the research strategies described previously.

**Table 6. Estimated Percentage of Fatalities Avoided in a Nuclear Incident via the Use of Postulated Radiation Medical Countermeasures**

<b>Washington, DC</b>	<b>Ground Release</b>			<b>Low Air Release</b>		
	<b>1 kT</b>	<b>10 kT</b>	<b>50 kT</b>	<b>1 kT</b>	<b>10kT</b>	<b>50 kT</b>
No treatment	0	0	0	0	0	0
Supportive care	15	6.3	0	18	10	0
Filgrastim* + Supportive Care	20	16	0	23	15	0
Maximal DRF**	92	56	0	92	22	0
<b>Heavy Brigade Combat Team</b>	<b>1 kT</b>	<b>10 kT</b>	<b>50 kT</b>	<b>1 kT</b>	<b>10kT</b>	<b>50 kT</b>
No treatment	0	0	0	0	0	0
Supportive care	13	12	0	11	14	0
Filgrastim* + Supportive Care	20	18	0	17	22	0
Maximal DRF**	93	69	0	81	26	0

\* Filgrastim is used here as an example because a DRF of its administration in conjunction with supportive care has been estimated.

\*\* The percent of fatalities avoided with a “Maximal DRF” countermeasure is equal to the percent of total estimated fatalities that are caused by radiation only, vice blast and thermal injuries.

The calculations in Table 6 of the effects of radiation medical countermeasures make several assumptions that may not be realistic. First, the analysis assumes that medical care can be delivered in 100% of cases with an essentially unlimited supply of medical care and doses. Second, this neglects the effects of combined injury—wounding and radiation injuries—which should increase fatality estimates.<sup>163</sup> Third, the ways in which radiation medical countermeasures could also affect blast or burn injuries is not considered. Lastly, although the LD<sub>50</sub> is a probabilistic value, it is used here deterministically.

Table 6 shows that approval of therapies yielding a DRF of less than two yield moderate improvements in fatalities from radiation. For example, if filgrastim and supportive care were available, treatment of IR-exposed patients following a 1 kT ground burst nuclear event would save 20% of total casualties, accounting for 2,650 civilians in

<sup>163</sup> The DRF for combined injury in mice is estimated to be 0.93. See Kiang et al., “Wound Trauma Increases Radiation-induced Mortality by Activation of iNOS Pathway and Elevation of Cytokine Concentrations and Bacterial Infection,” *Radiation Research* 173 (2010): 319–32.

DC or 18 military personnel in a HBCT-O scenario. Both of these are significant savings. However, to have a large impact on overall fatalities, saving over 90% of patients or 11,800 civilians or 83 military personnel, would require a DRF of 161. While this is a lofty goal indeed, it also indicates that there is ample room for improvement if a very novel therapy were discovered.



## **5. Setting Priorities for Radiation Medical Countermeasure Research**

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While the ultimate goal of a radiation medical countermeasure is to protect and sustain life to the best degree possible during and following a radiation incident, the R&D required to achieve a medical countermeasure capable of providing a significant survival advantage is expensive, time-consuming, and has a high risk of failure. To date, the only FDA-approved treatment for ARS is supportive care, which will provide against 1.5 LD<sub>50</sub>s of IR. In IDA's illustrative example of a nuclear weapons detonation in Washington, DC or an HBCT-O, this results in a fatality reduction of between 6 and 18%. Filgrastim is currently stockpiled in the SNS by the CDC, and if filgrastim were also used in conjunction with supportive care in these scenarios, it could protect against 1.88 LD<sub>50</sub>s of IR, leading to a fatality reduction of between 15 and 23%.

The primary targets of countermeasure research have generally focused on IR doses that cause lethality due to the HP subsyndrome, though there is some early-stage research into therapies for the GI subsyndrome. Many of the therapeutic advances using leukocyte growth factors as targets for treating the HP subsyndrome originated in the scientific community's understanding of radiation therapy, chemotherapy-induced myelosuppression, and bone marrow transplants. Leukocyte growth factors and other cytokines have been investigated for their potential to stimulate hematopoiesis from hematopoietic stem cells and their progenitors following bone marrow ablation and chemotherapy-induced myelosuppression,<sup>164</sup> which are models similar to the HP subsyndrome of ARS. This does not necessarily mean that leukocyte growth factors would be the most effective treatments for ARS in general, and there is research in various stages of development suggesting new targets for ARS countermeasures. However, since cytokines have a historical presence in the clinic for indications similar to ARS, leukocyte growth factors are currently the therapies closest to achieving FDA approval, and other cytokines remain good candidates for drug repurposing to treat ARS.

It is important to note that initial insight into hematopoiesis originated from the Cold War fear of nuclear weapons, and the desire to protect military personnel and

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<sup>164</sup> The first clinical trials for filgrastim (G-CSF) were in the late 1980s. For a review see K. Welte, "Discovery of G-CSF and Early Clinical studies," in *Twenty Years of G-CSF*, ed. G. Molineux, M. Foote, and T. Arvedson (Basel: Springer AG: 2012).

civilians from total body irradiation (TBI).<sup>165</sup> In the early 1960s, with this motivation (and funding from the Canadian Department of National Defence), Canadian scientists Ernest McCulloch and James Till discovered the first stem cell, the hematopoietic stem cell, which they determined gave rise to other hematopoietic stem cells, as well as red blood cells, white blood cells, and platelets.<sup>166</sup> These advances in the fundamental understanding of hematopoiesis and later the HP subsyndrome underscore the importance of investments in research other than direct drug development. With the next focus for countermeasure research being in GI ARS, a greater understanding of its pathology, both in its similarities to IBD and in order to develop HTS assays, would be steps in gaining necessary knowledge to discover treatments for GI ARS, allowing for protection against doses of IR greater than two LD<sub>50</sub>s. Several INDs seeking approval for IBD are also attempting to receive a secondary indication for ARS.

In addition to describing various drug development and research strategies for countermeasures and considering them in terms of their proximities to FDA approval, IDA's research also stresses the need for developing a qualified animal model in which to study countermeasure efficacy. This is not only with the goal of streamlining the approval process, but it also provides a similar metric so that countermeasures can be properly compared with each other. It is unlikely that DOD would require multiple countermeasures with the same therapeutic targets and similar efficacies.

In closing, the recommendations of IDA's research are as follows:

1. Invest greater efforts in drug repurposing to save cost, time, and risk in drug development. Traditional drug development avenues can still be supported, but they should not be considered the only approach available.
2. Due to the cost and effort of approval and stockpiling treatments, once the first countermeasure is approved that protects against a given dose, there is little benefit to financing the approval of another if it does not have a considerable advantage.
3. Support research into IBD and other GI disorders that have mechanistic overlap with GI ARS. Additionally, support dual indications for INDs that could treat GI ARS in addition to IBD.

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<sup>165</sup> E. Strauss, "2005 Albert Lasker Basic Medical Research Award, Award Description, Ernest McCulloch and James Till," last accessed August 28, 2014, [http://www.laskerfoundation.org/awards/2005\\_b\\_description.htm](http://www.laskerfoundation.org/awards/2005_b_description.htm).

<sup>166</sup> E. A. McCulloch and J. E. Till, "The Radiation Sensitivity of Normal Mouse Bone Marrow Cells, Determined by Quantitative Marrow Transplantation into Irradiated Mice," *Radiation Research* 13 (1960):115–25; J.E. Till, and E.A. McCulloch, "A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells," *Radiation Research* 14 (1961): 213–222; and A.J. Becker, E.A. McCulloch, and J.E. Till, "Cytological Demonstration of the Clonal Nature of Spleen Colonies Derived from Transplanted Mouse Marrow Cells," *Nature* 197 (1963): 452–4.

4. Support the development and implementation of GI ARS HTS assays. These can be used to screen novel molecular entities, new variants of lead compounds, and libraries of drugs with prior FDA indications. Additionally these might help in identifying new combinations of therapies.

As many potential countermeasures discussed here have DRFs within the range of supportive care (1.5) or filgrastim and supportive care (1.88), an advantage in efficacy alone is unlikely. Some may have other benefits, and these other benefits should be considered in addition to efficacy in prioritization. However, if significant advances are to be made in the efficacy of radiation medical countermeasures, therapies with very novel mechanisms of action should be investigated. Furthermore, basic research into the underlying physiological, cellular, and molecular changes that occur following IR exposure could potentially lead to new therapies with much greater efficacies. Funding priorities will likely require a multi-pronged approach that balances the requirements and needs of the U.S. Government.



## Appendix A

### Ionizing Radiation

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#### **Ionizing Radiation (IR)**

IR can be characterized as either particulate or electromagnetic, though the wavelengths of the electromagnetic spectrum that are ionizing are of such high energy that these wavelengths show particle-like behavior, as well, and are sometimes described as such. Electromagnetic IR includes x- and gamma-rays, and particulate IR includes neutrons and alpha particles. X- and gamma-rays are considered functionally synonymous in their mechanisms of damage to and effects on the body. They are both used interchangeably and are the most common IR sources used to induce ARS in experimental models. However, since neutrons are often released during a nuclear explosion, mixed gamma-rays and neutrons are sometimes also studied. Less commonly, neutrons are the sole IR source in experiments. Alpha particles require deposition into or onto the body to initiate health consequences, which is a less likely occurrence and therefore infrequently studied. Furthermore, many alpha particles can be removed from the body with decontamination therapies. Experiments and models using (1) x- or gamma-rays, (2) mixed gamma-rays and neutrons, (3) neutrons, or (4) alpha particles should all be considered separately, since the mechanisms of insult and effects on the body are different as a result of exposure to each of the four IR sources.

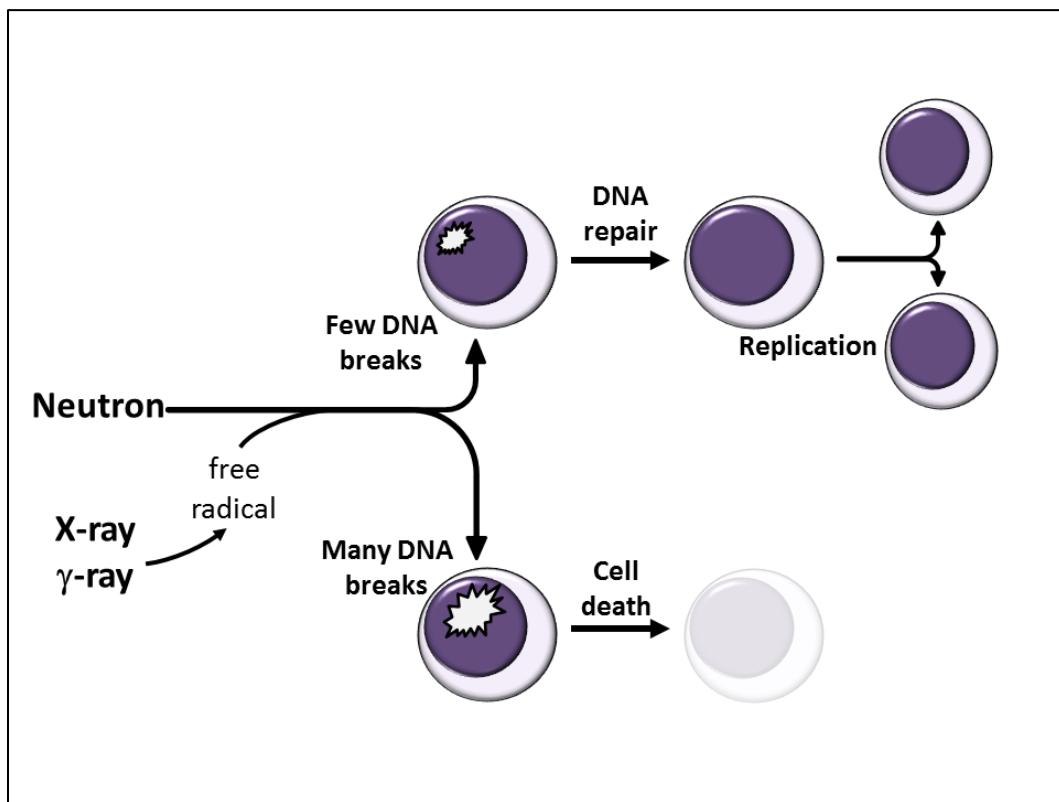
#### **IR-induced DNA Damage**

The IR-damage pathway is shown in Figure A-1. The human response to IR begins with its fundamental mechanism of cellular damage. X- and gamma-IR indirectly damage the cell by producing free radicals that can break deoxyribonucleic acid (DNA) strands, though other parts of the cell are susceptible as well. Neutrons and alpha particles more often act directly on the DNA, which, in theory, makes this type of radiation resistant to medical countermeasures that are free radical scavengers. Effects from damage to anything other than DNA are not considered here, nor are the “bystander effects,”<sup>1</sup> as the understanding in these fields is nascent and so far not a major subject of countermeasure research. However, it is likely that both damage to non-DNA molecules and bystander

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<sup>1</sup> “Bystander effects” are those physiological consequences in a cell when that cell itself was not exposed directly to IR, that is, by IR directly traversing the cell.

effects contribute to ARS and may also be affected by the countermeasures discussed next.



**Figure A-1. IR-Damage Pathway in Cells**

### Cellular Response to IR-induced DNA Damage

Every diploid human cell has about two meters of DNA within its nucleus.<sup>2</sup> As the dose of IR increases, the DNA breaks in more places. About every third of a nanometer across the entire two meters of DNA is a location for the DNA to break, potentially six billion break points. In normal cellular life, the DNA incurs a small number of breaks, and the cell has sophisticated repair machinery to return the DNA to its original state. However if the DNA is broken in too many locations, it cannot be repaired faithfully, and the cell might behave, grow, or reproduce aberrantly, eventually leading to cancer. To

<sup>2</sup> One DNA base is approximately  $3.4 \times 10^{-10}$  of a meter long, and there are about  $6 \times 10^9$  bases on the 46 chromosomes within a diploid human cell. Therefore, each cell contains about 2 meters of DNA. See A. Annunziato, "DNA Packaging: Nucleosomes and Chromatin," *Nature Education* 1 (2008): 26 for a good summary of how two meters of DNA is wound and packed into a human cell with an average diameter one million times smaller.

avoid this outcome, cells are programmed to die if their DNA is too badly damaged that the cell cannot repair itself correctly.

Following exposure to a low IR dose, such as that incurred in a routine medical x-ray, cells can often recover utilizing their innate DNA repair pathways. A small number of cells may die, though they are quickly replaced with little consequence to the organism. However, if the body receives a pathological dose of IR, cells cannot repair themselves, and they die either through mitotic catastrophe or apoptosis—two related cell death mechanisms.<sup>3</sup> It is this massive cell death by mitotic catastrophe of the hematopoietic (HP) and gastrointestinal (GI) stem cells that usually leads to sequelae and death of the organism. Apoptosis and mitotic catastrophe of other cell types also contribute to ARS. Since all of the differentiated (non-stem) cells of the HP and GI systems have limited lifespans, these systems are in essence regenerated every 4 to 120 days, with the HP and GI stem cells being the sources of new cells for these systems. When too many stem cells die due to IR exposure, the systems are eventually depleted of cells, repopulation cannot occur, and the body ceases to function in their absence.

All cells can be damaged by IR, but the effects of unrepairable DNA strand breaks may not affect the cell too greatly until it attempts to replicate its DNA in anticipation of a cell division. DNA replication requires the entire DNA code to be read and reproduced, and if the strand backbone is broken the replication machinery stops and cannot continue to read the code until repair occurs. Alternately, the cell may die if it cannot repair its DNA. IR-induced effects on cell division are directly related to the damage incurred at the DNA level.

Cells have various reproductive potential, and some tissues have a high rate of turnover of cells. Sensitivity to radiation is directly correlated with higher levels of mitotic activity, a property that was originally observed by Bergonie and Tribondeau in the mid-twentieth century.<sup>4</sup> The more rapidly proliferating cells die earlier than quiescent cells in response to IR. Casarett later described categories of mammalian tissue radiosensitivity based on this property of early cell death.<sup>5</sup> As shown in, earlier cell death in response to radiation and greater sensitivity to lower doses of IR are properties of relatively undifferentiated, regularly dividing cells. These include stem cells such as HP and GI crypt stem cells. Partially differentiated cells, such as lung, liver, and kidney cells

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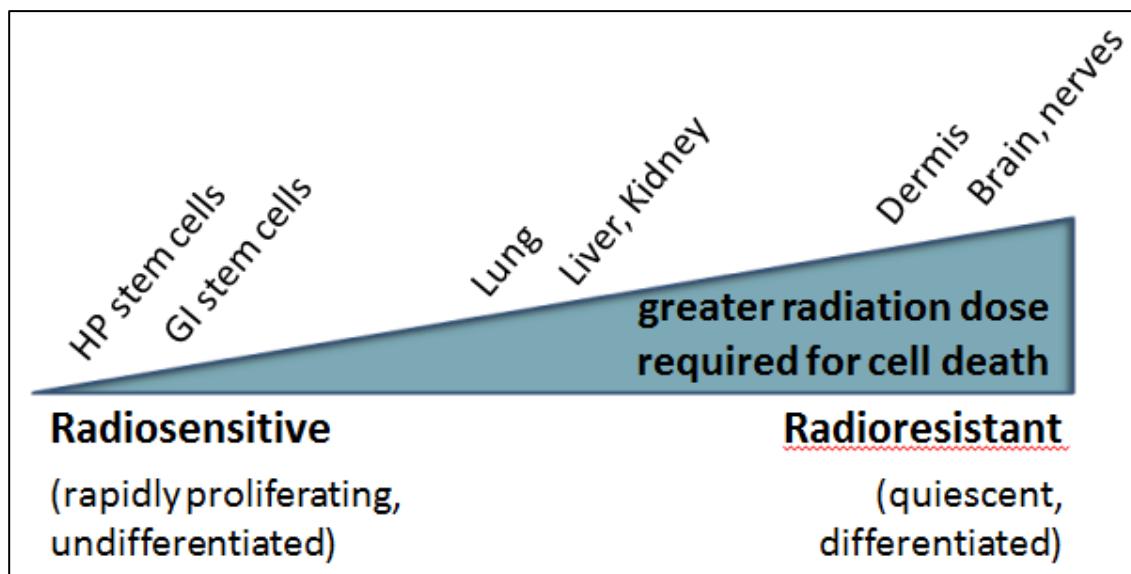
<sup>3</sup> For further information on the ongoing discussion of the similarities and differences between apoptosis and necrosis see Vitale et al., “Mitotic Catastrophe: A Mechanism for Avoiding Genomic Instability,” *Nature* 12 (2011): 1–8. The nuances of these two types of cell death may be important in targeting these pathways for future ARS therapies.

<sup>4</sup> J. Bergonie, and L. Tribondeau, “Interpretation of Some Results of Radiotherapy in an Attempt at Determining a Logical Technique of Treatment,” *Radiat Res* 11(1959): 587–8.

<sup>5</sup> P. Rubin, and G.W. Casarett, *Clinical Radiation Pathology*, Vol I (Philadelphia, PA: W.B. Saunders 1968).

are moderately sensitive. Terminally differentiated cells, such as dermis and neurons, are quite insensitive to IR-induced cell death.<sup>6</sup>

As Figure A-2 shows, the GI and HP systems are both acutely affected by IR, and they both require frequent repopulation for proper function. The epithelial cells of the intestinal villi are repopulated every four to five days.<sup>7</sup> The HP system repopulates various cell components at different rates, which are on average longer than that of the intestine. However, HP cells are more sensitive to lower doses of radiation, and the HP effects occur at lower doses of radiation than the GI tract. However, at higher IR doses where both GI crypt stem cells and HP stem cells are sensitive, patients succumb to death from the loss of the GI system before the HP system is depleted due to the GI system's faster repopulation kinetics.



**Figure A-2. Rapidly Proliferating, Undifferentiated Cells Are Most Sensitive to IR**

## Acute Radiation Syndrome (ARS)

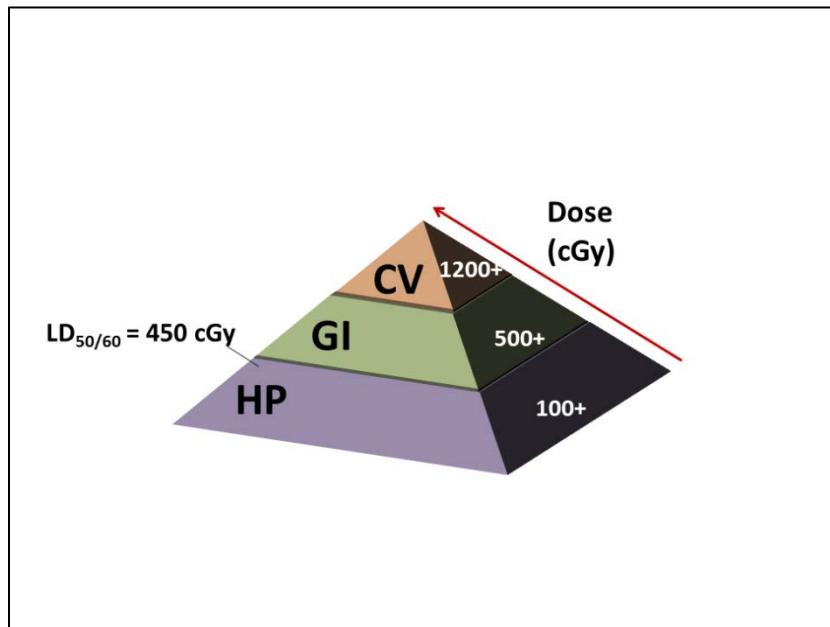
ARS is the cumulative manifestation of the human body's complex responses to high doses of IR. ARS is a complicated medical situation, and is comprised of three separate, yet related, subsyndromes—the HP, GI, and cerebrovascular (CV) subsyndromes. While these three subsyndromes were originally thought to be distinct facets of ARS, the subsyndromes, in fact, have enough overlap with each other that their

<sup>6</sup> E.J. Hall, and A.J. Giaccia, *Radiobiology for Radiologist*, 7th ed. (Philadelphia, PA: Lippincott Williams & Wilkins, 2012).

<sup>7</sup> L.G. Van der Flier, and H. Clevers, "Stem Cells, Self-renewal, and Differentiation in the Intestinal epithelium," *Annu Rev Physiol* 71 (2009): 241–60.

interdependency is often described as the interrelated multi-organ failure (MOF) or multi-organ dysfunction syndrome (MODS).<sup>8</sup>

The doses at which the various subsyndromes arise are related to the relative sensitivities of the stem cells. HP stem cells are most radiosensitive, followed by GI stem cells. Therefore, the HP subsyndrome occurs at a lower dose of IR. CV subsyndrome is not well understood, but it occurs at a very high dose of IR. This is shown in Figure A-3. This paper assumes that ARS generally begins at doses of 125 cGy or more.<sup>9</sup>



Note: The doses given are approximate, and different references might have slightly different ranges.

**Figure A-3. ARS Subsyndromes Emerge at Different Doses of IR Partially Reflective of Their Respective Stem Cell Radiosensitivities**

For the purposes of this paper, unless otherwise noted, the centiGray (cGy) will be used as the dose units, and the dose will be expressed as free-in-air (FIA). Fractionated doses will not be considered, and only total body irradiation (TBI) without shielding will be discussed, with the exception of a limited discussion of potential countermeasures for the GI syndrome where experiments are typically performed with some bone marrow protection to allow for better isolation of the GI syndrome. Also, the radiation is assumed

<sup>8</sup> H. Dörr and V. Meineke, “Acute Radiation Syndrome Caused by Accidental Radiation Exposure—Therapeutic Principles,” *BMC Med* 9 (2011): 126–31; and J. P. Williams and W. H. McBride, “After the Bomb Drops: A New Look at Radiation-Induced Multiple Organ Dysfunction Syndrome (MODS),” *Int J Radiat Biol* 87 (2011): 851–868.

<sup>9</sup> AMedP-8(C) uses 125 cGy as the lower bound total body irradiation (TBI) dose at which symptoms of ARS appear.

to be all x- or gamma-IR. However, note will be made of the efficacy, if it is known, of the countermeasure against neutrons or mixed radiation sources.

The more radiosensitive subsyndromes persist after subsequent subsyndromes arise. That is, although symptoms of the GI subsyndrome may be apparent, the HP subsyndrome still exists, and the higher dose that led to GI symptoms also exacerbates the HP subsyndrome. This is reflected in the pyramidal shape of the dose response of subsyndromes in Figure A-3.

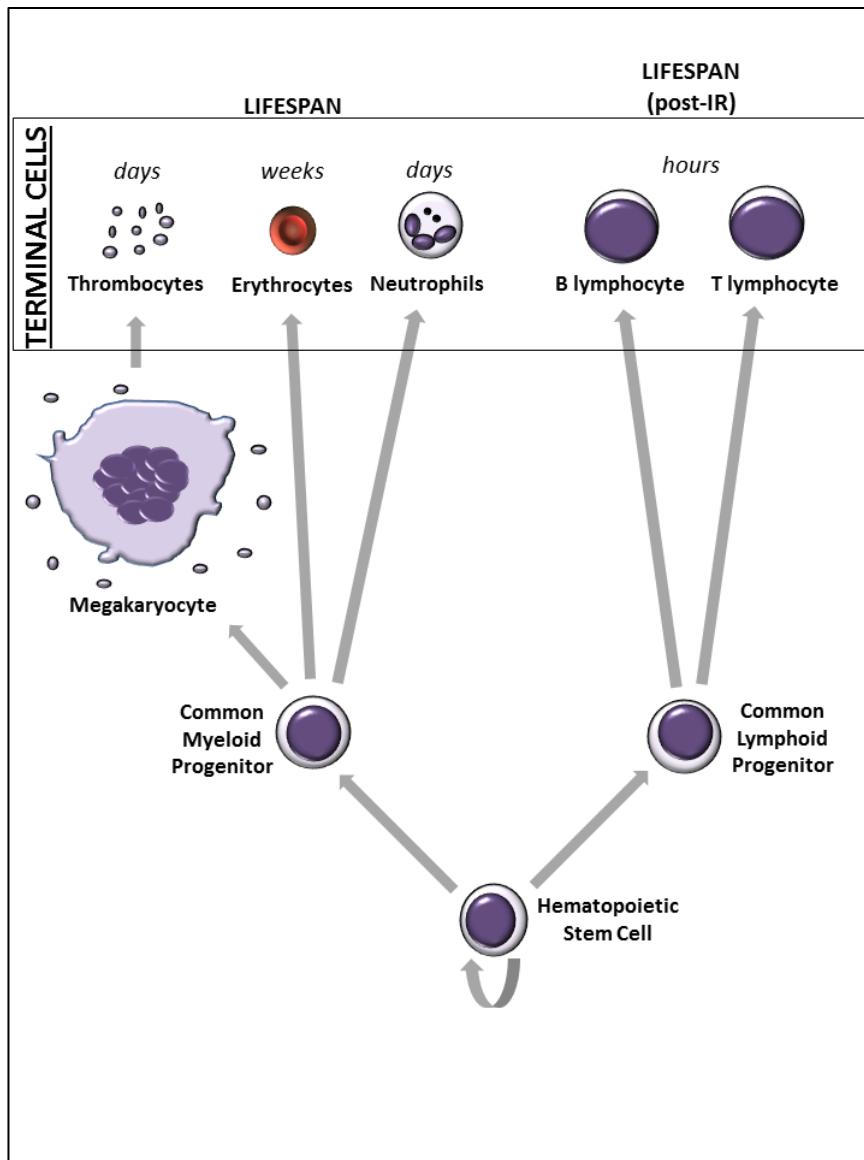
## **ARS Subsyndromes**

### **5. Hematopoietic (HP) Subsyndrome**

The HP system in Figure A-4 repopulates by hematopoiesis, which takes place in the bone marrow, blood, and periphery. The HP stem cells, which are very sensitive to IR, have more or less limitless potential for self-renewal or differentiation. In self-renewal, they create copies of themselves that can in turn self-renew or differentiate. In differentiation, HP stem cells become either myeloid or lymphoid progenitors. Once the cells have differentiated, they cannot de-differentiate or return to the multipotent stem cell status. Lymphoid progenitors can self-renew to create more lymphoid progenitors or differentiate to B and T lymphocytes, and myeloid progenitors can self-renew or differentiate into megakaryocytes (which make thrombocytes), erythrocytes, or neutrophils.<sup>10</sup> Platelets, erythrocytes, and neutrophils all have a set lifespan and cannot reproduce or differentiate further. Some lymphocytes can further divide and differentiate if activated by an antigen, and others are already terminally differentiated.

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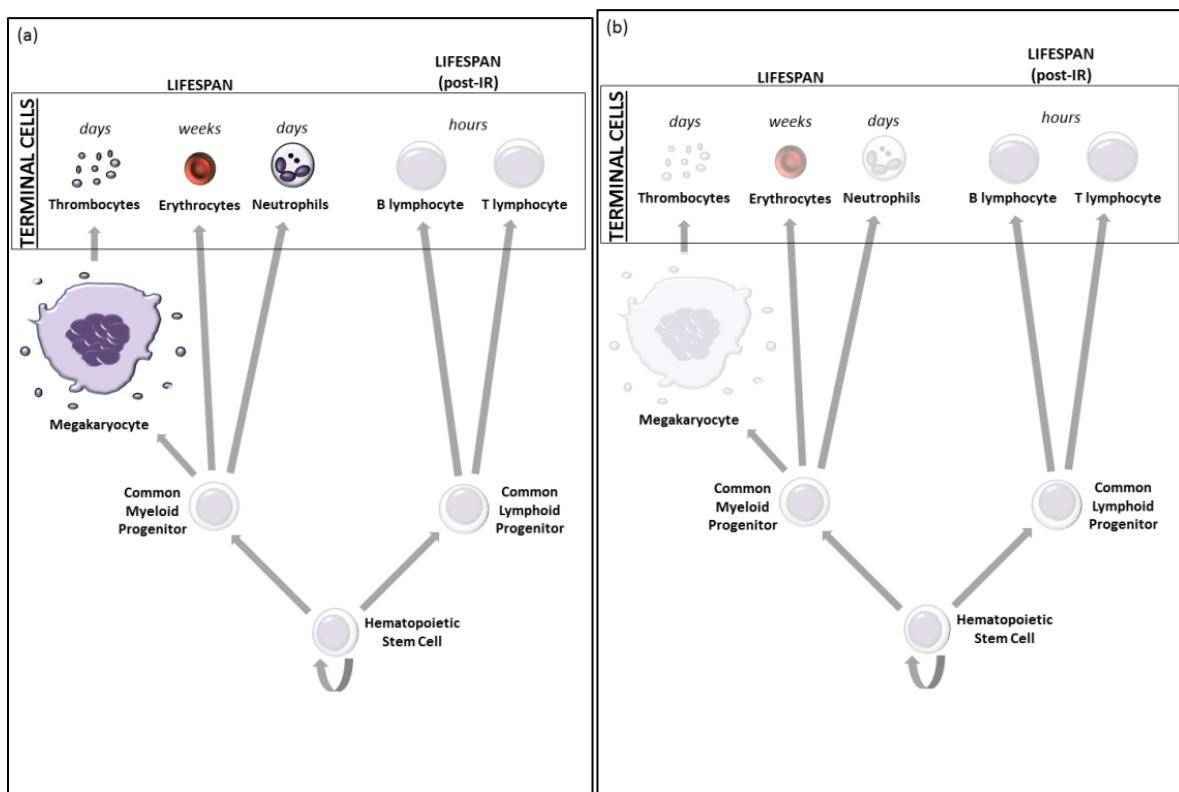
<sup>10</sup> Both lymphoid and myeloid progenitors can differentiate into other types of cells that are not discussed here because they are not commonly measured following IR.



**Figure A-4. Hematopoiesis Showing HP Stem Cells Giving Rise to Progenitors Which Further Differentiate into Terminally Differentiated Cells**

In response to IR exposure, the HP stem cells are destroyed and can no longer reproduce or differentiate. In contrast, platelets, erythrocytes, and neutrophils are rather insensitive to IR. However, due to their limited lifespans their levels decline in the absence of stem cells, and there is no repopulation if the IR dose is high enough. If the IR dose does not ablate all stem cells, it is possible to repopulate the HP system without an HP stem cell transplant, but this takes time because the stem cell population is reduced and needs to self-renew in addition to repopulation. Lymphocytes disappear rapidly following IR: although they are not rapidly dividing, they are an unconventional cell type that happens to be very radiosensitive. Within one to two days lymphocyte levels drop to their nadirs. Figure A-5(a) shows the effects of IR on the HP system within a few days of

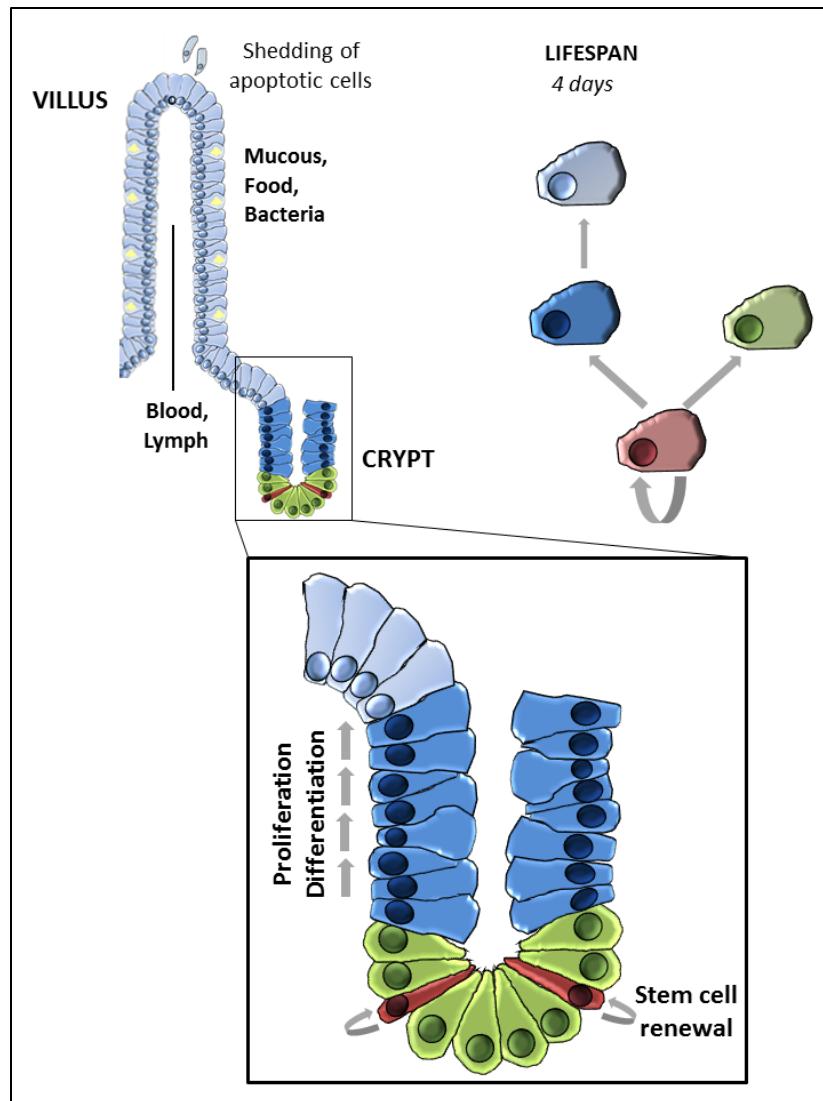
exposure (compare this to A-4), and Figure A-5(b) shows the HP system within a few weeks of IR exposure. Of note is the continued, though reduced, presence of red blood cells. Owing to the long lifespan of erythrocytes, severe anemia is not usually a significant problem following IR. Repopulation of the HP system begins between 30 and 40 days post-irradiation.



**Figure A-5. HP Cell Loss Following IR at Approximately (a) 2 d Post-exposure and (b) 14 d Post-exposure Following an IR Dose at the LD<sub>50/60</sub>.**

## 6. Gastrointestinal (GI) Subsyndrome

The GI system repopulates every four to five days. Figure A-6 shows the population dynamics of the cells in the villi. Self-renewing intestinal crypt stem cells (red) divide or differentiate to form paneth cells (green), which remain in the crypt, or proliferative progenitors (dark blue), which transverse up the crypt. The proliferative progenitors then differentiate further (light blue) and move into the villus. Lastly, at the tip the oldest epithelial cells undergo apoptosis and are shed from the villus. They are replaced by newer epithelial cells.



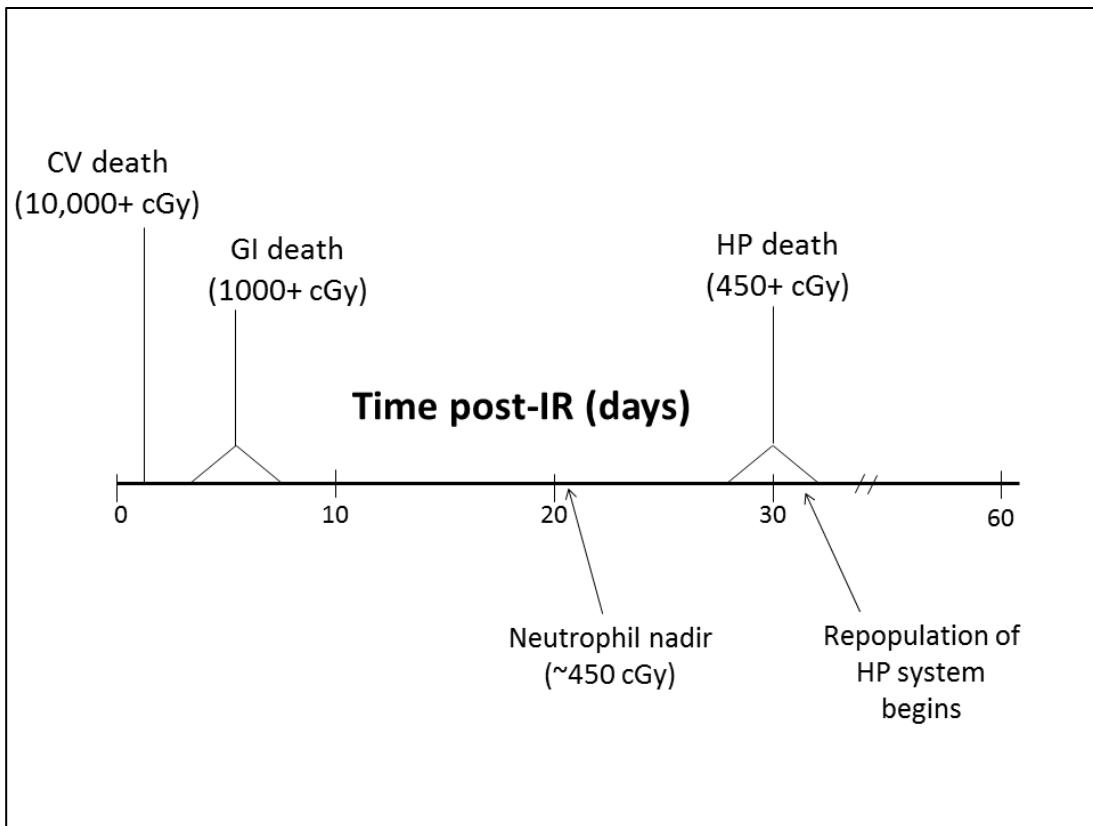
**Figure A-6. GI Epithelial Cell Population and Its Turnover in the Intestines**

In response to IR, the crypt stem cells and progenitors are damaged, though the terminally differentiated cells remain relatively unaffected. However, due to their short lifespan, the epithelial cells continue to die and be shed from the villus without replacement. The villi shorten until they eventually disappear. As the epithelial lining becomes discontinuous, bacteria escape into the blood leading to sepsis, which is further exacerbated by the compromised immune system.

At lower doses of IR, less severe symptoms are observed as more mild and moderate cytopenias. The lower GI system is relatively unaffected and the gut remains intact. However, at higher doses of IR, more severe symptoms are observed including the onset of severe GI symptoms and sepsis.

## The Timings of Death from ARS Subsyndromes

ARS has a relatively slow progression, shown over time in Figure A-7, and survival is usually measured at the end of 30 or 60 days for non-human primates (NHP) or humans, respectively. Median lethal dose ( $LD_{50}$ ) in ARS is usually defined as the  $LD_{50/30}$  or  $LD_{50/60}$ , which is the IR dose at which half of the NHP die after 30 days or half of humans die after 60 days, respectively. Unsurprisingly, at lower doses of IR, death occurs later than at higher doses. If the dose of IR is low enough that only the HP subsyndrome is apparent (450 cGy or less), then the neutrophil nadir should occur around 20 days post-IR. If the HP system can repopulate from its remaining HP stem cells, this usually begins just after a month post-IR. This leaves a window of ten or more days during which an individual is incredibly susceptible to infection, and it is during this interval that one can succumb to complications from sepsis. At higher doses of IR, the GI subsyndrome occurs in conjunction with HP subsyndrome. This leads to an earlier neutrophil nadir, perforation of the epithelial lining of the gut, leakage of gut contents into the blood, and death from sepsis occurs at a much earlier time, usually in less than ten days. At very high doses of IR, around 10,000 cGy, death from the CV subsyndrome occurs within two days. The shorter lifespan of the epithelial cells of the GI system compared to that of the terminally differentiated HP cells contributes, at least in part, to the shorter time to death from the GI subsyndrome.



Note 1: "CV death" and "GI death" do not imply that the underlying HP and GI subsyndromes or HP subsyndrome, respectively, do not also have a role in the death at these higher doses. However, the primary cause of death will be from the CV or GI symptoms.

Note 2: Times of death are approximate and from E.J. Hall and A.J. Giaccia, *Radiobiology for Radiologist*, 7th ed. (Philadelphia, PA: Lippincott Williams & Wilkins, 2012).

**Figure A-7. Timeline of ARS and Death from Subsyndromes**

## Phases of ARS

ARS is separated into three primary phases, in addition to possible recovery, up to the first 60 days following exposure. The phases of ARS are the prodromal phase, latent phase, and the manifest phase. Delayed effects, which are not included in ARS by definition, could follow these first 60 days of ARS, and may include fibrosis of the lung, kidney, neuromusculature, etc, as well as lung pneumonitis and others. Recovery occurs once the systems affected in ARS have recovered.

Furthermore, based on the linear non-threshold (LNT) model for delayed effects used in Biological Effects of Ionizing Radiation (BEIR) VII,<sup>11</sup> it is estimated that there is

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<sup>11</sup> National Research Council (NRC), *Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2* (Washington, DC: National Academies Press, 2006).

an increased risk of lifetime fatal cancer beginning at 0.05 cGy,<sup>12</sup> which would be a delayed effect. The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) estimates the lifetime risk of IR exposure-induced fatal solid cancer following 10 cGy is 0.36-0.77%.<sup>13</sup>

Figure A-8 shows the three phases of ARS with increasing severity, and the corresponding time of onset of symptoms and blood profiles. Figure A-8 combines information from *Allied Medical Publication-6(B)* [AMedP-6(B)],<sup>14</sup> *AMedP-8(C)*,<sup>15</sup> the Medical Treatment Protocols for Radiation Accident Victims (METREPOL),<sup>16</sup> and Radiation Emergency Medical Management (REMM),<sup>17</sup> including the incorporation of symptom severities from *AMedP-8(C)* and HP system severity levels (H1 through H4) from METREPOL.

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<sup>12</sup> Military Medical Operations, , “Medical Management of Radiological Casualties, 3rd ed.” (Bethesda, MD: Armed Forces Radiobiology Research Institute, 2009).

<sup>13</sup> United Nations Scientific Committee on the Effects of Ionizing Radiation, “UNSCEAR 2006: Effects of Ionizing Radiation, Volume I, Annex A: Epidemiological Studies of Radiation and Cancer” ( New York: United Nations, 2008).

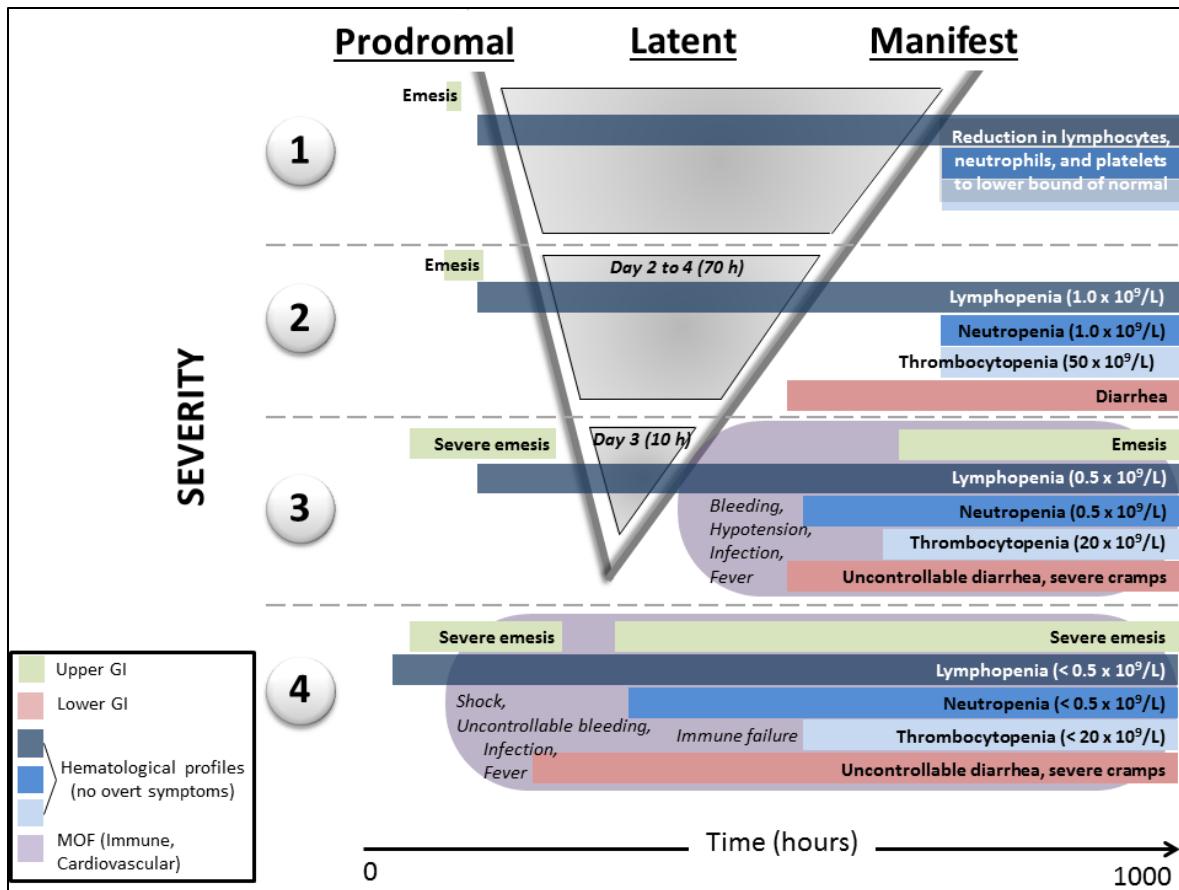
According to the Surveillance, Epidemiology, and End Results [SEER] Program, the lifetime risk of developing fatal cancer in the US is 20.93%—see National Cancer Institute, Surveillance Research, Cancer Control and Population Sciences, “SEER Cancer Statistics Review 1975–2010, Table 1.18: Lifetime Risk (Percent) of Dying from Cancer by Site and Race/Ethnicity, Both Sexes, Total U.S., 2008-2010,” <http://surveillance.cancer.gov/devcan>, accessed Mar 5, 2014. UNSCEAR statistics are applicable to 2006 Chinese, Japanese, Puerto Rican, U.S., or United Kingdom populations. The SEER statistics are for the U.S. population.

<sup>14</sup> North Atlantic Treaty Organization (NATO), *AMedP-6(B): NATO Handbook on the Medical Aspects of NBC Defensive Operations*, STANAG 2500, July 1994.

<sup>15</sup> C. A. Curling et al., *Addenda to Allied Medical Publication 8, “NATO Planning Guide for the Estimation of Chemical, Biological, Radiological, and Nuclear (CBRN) Casualties” (AMedP-8(C)) to Consider the Impact of Medical Treatment on Casualty Estimation*, IDA Document D-4466 (Alexandria, VA: IDA, May, 2013).

<sup>16</sup> T. M. Fliedner, I. Friesecke, and K. Beyer, “Medical Management of Radiation Accidents—Manual on the Acute Radiation Syndrome” (British Institute of Radiology, 2001).

<sup>17</sup> U. S. DHHS, “Radiation Emergency Medical Management: Guidance on Diagnosis and Treatment for Health Care Providers,” August 30, 2013, <http://www.remm.nlm.gov>.



Note 1: L, liter; h, hour.

Note 2: The latent period for severity level 1 is the end of the syndrome. While the blood cell counts are lower than normal, they are not considered particularly pathological, and there is usually no obvious manifestation of ARS after the prodromal phase in level 1. Severity level 4 has no latent period.

**Figure A-8. Three Phases of ARS and Corresponding Symptomatology and Hematological Profiles**

A distinction should be drawn regarding symptoms and blood profiles. The hematological counts of lymphocytes, neutrophils (granulocytes), and thrombocytes (platelets) are not themselves *symptoms*. However, they are markers of the severity of radiological insult to the body. For the purpose of medical treatment following IR and chemotherapy, which can cause similar pancytopenia, measurements of these three cell types, along with red blood cells (RBCs) and other hematological measures, can indicate treatment options and severity. Many overt symptoms such as fever and infection are a direct result of the clinically measurable pancytopenia.

### 1. Prodromal Phase

The prodromal phase is characterized by the appearance of acute symptoms beginning between 20 minutes and 3 hours following IR exposure. Emesis is the most common prodromal symptom, though it does not occur in everyone. Emesis can range

from mild to severe, and time to onset is inversely proportional to the dose received. It is unclear why vomiting occurs, but it is thought to originate from the release of neurotransmitters at the vagus nerve terminal. This is further supported by the efficacy of serotonin receptor antagonists at treating the emetic reaction.

Lymphopenic nadir also occurs during the prodromal phase, though the effects of it are usually noticed in later phases with the onset of infection in conjunction with other blood cell losses. However, at very high doses of IR, the effects of massive infection due to leukopenia are evident (see “Severity 4” in Figure A-8).

## **2. Latent Phase**

The latent phase is a relatively asymptomatic period of time in ARS where the emesis of the prodromal phase subsides and before the manifest symptoms appear. While the patient may appear to be recovering, the blood and GI cells continue to die off during this period without being replaced. When the cells reach a critically low level, the manifest stage begins with its overt symptoms.

The length of the latent phase is inversely proportional to the dose received. That is, those receiving low doses have a longer latent phase than those receiving higher doses. (This is represented in the triangular shape of the latent phase in Figure A-8.) In patients receiving very high doses of radiation, no observable latent phase occurs.

## **3. Manifest Phase**

The manifest phase begins when the GI and HP cells reach a low enough level for diarrhea, bleeding, blood pressure reduction, and infection to occur. Vomiting can also occur in the manifest phase if the IR dose received is high enough. The blood cell nadirs are observed during the manifest phase except the lymphocyte nadir, which occurs during the prodromal phase.

## **4. Delayed Effects**

Although delayed effects from IR occur 60 days after exposure, they are still often considered part of ARS. In addition to cancer, they can include fibrosis, pericarditis, and pneumonitis as well as other long-term effects.

Since all of the therapies used in supportive care are FDA-approved and used to treat conditions that can also be found in other illnesses (e.g., diarrhea), their safeties and efficacies are well-defined. Precautions, however, need to be taken in using these therapies because ARS is a complicated series of conditions, and the side effects of some therapies could aggravate other ARS-related conditions. For example, using aspirin as an analgesic for pain associated with ARS might be contraindicated because of ARS-induced thrombocytopenia.

## Appendix B

### Defining Supportive Care for ARS

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There is no standard definition of supportive care for ARS, so not every individual exposed to IR will receive the same supportive care. This is unsurprising, as the elements of supportive care should directly address the symptoms experienced by the patient, which are unique to the patient, rather than all of the potential symptoms that could manifest in ARS. Further contributing to the heterogeneity in defining supportive care is that not all elements of supportive care are available at all locations. Even when receiving similar doses of IR, each individual can experience an array of symptoms of varying severities. Maintaining a well-stocked supportive care toolkit of therapies provides flexibility in the treatments of each specific patient. Supportive care can include anti-emetics, GI tract protection, analgesics, brain edema therapies, adapted nutrition, antibiotic treatment, anti-diarrheals, blood component substitution, and side effects management.

In the absence of blood component substitution, the terminally differentiated cells remain low until the endogenous stem cells can repopulate the HP system (Figure A-7). However, by managing symptoms, infection, and nutrition, the prognosis can often be improved. While blood component replacement, such as platelets, may be important to the successful administration of supportive care, these terminally differentiated constituents have their set lifespans and are only a temporary measure until the repopulation capabilities of the HP system can occur. In addition to platelets, erythrocytes are another common blood component for replacement, though anemia is less common than other blood cytopenias following ARS.

HP stem cell transplant can become necessary if the stem cell population is so badly damaged that reconstitution of the HP system cannot be achieved from the remaining stem cell population. However, graft-versus-host disease (GvHD) or rejection is always a possibility. Fliedner et al. described a profile of blood cell changes following ARS that suggests an irreversible marrow damage that would necessitate a HP stem cell transplant.<sup>1</sup> Understanding these changes could avoid unnecessary stem cell transplants, as they have risks to both patient and donor and require immunosuppressive therapy to

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<sup>1</sup> T.M. Fliedner et al., “Pathophysiological Principles underlying the Blood Cell Concentration Responses Used to Assess the Severity of Effect after Accidental Whole-Body Radiation Exposure: An Essential Basis for an Evidence-Based Clinical Triage,” *Exp Hemat* 35 (2007): 8–16.

prevent GvHD.<sup>2</sup> Alternatives to autologous and allogenic bone marrow transplants are using mobilized progenitors from peripheral blood or donated umbilical cord blood. These three methods of stem cell transplant therapy have their associated risks and benefits, which are briefly reviewed in the METREPOL manual.<sup>3</sup>

The first large-scale use of supportive care occurred following the 1986 Chernobyl accident. Data on patient prognoses and outcomes from Chernobyl<sup>4</sup> versus the students and instructors housed in a school during the Nagasaki bombing of 1945<sup>5</sup> have been used to estimate the effects of supportive care in humans. Certain limitations exist when comparing the Chernobyl and Nagasaki datasets, such as the mean ages (31 years versus 22.4 years, respectively), sex (all male versus male and female), and initial health of the populations (fit versus undernourished). Furthermore, it is likely that there were a greater mean dose and a wider range of doses experienced in the Nagasaki patients compared to Chernobyl, which can be inferred based on several factors including the earlier time of death of the Nagasaki cohort suggestive of GI syndrome. Another confounding factor was that the two probit slopes from the datasets were significantly different. Also, the radiation in the Nagasaki cohort was likely mixed fission-neutron-gamma, while the IR from the Chernobyl accident was primarily gamma.<sup>6</sup> Despite the problems in comparing the two datasets, Anno et al. attempted to estimate a dose reduction factor (DRF)<sup>7</sup> for supportive care, estimating that at the LD<sub>50/60</sub>, the DRF for supportive care would be about 2.0<sup>8</sup> using Chernobyl and Nagasaki data.

A second seminal study in the effects of supportive care on the dose response relationship of ionizing radiation was reported by MacVittie et al. in 1991 in canines. Supportive care in this study consisted of antibiotics, intravenous fluid support, and

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<sup>2</sup> Dr. James Armitage discusses barriers to transplantation in ARS on pp. 486–8 of N. Daniak, J. K. Waselenko, J. O. Armitage, T. J. MacVittie, and A. M. Farese, “The Hematologist and Radiation Casualties,” *Hematology Am Soc Hematol Educ Program* (2003): 473–96.

<sup>3</sup> Fliedner et al., “Medical Management of Radiation Accidents— Manual on the Acute Radiation Syndrome.”

<sup>4</sup> A. Laupa, and G. H. Anno, *Chernobyl Accident Fatalities and Causes*, DNA-TR-89-45 (Washington, DC: Defense Nuclear Agency, 1990).

<sup>5</sup> S. G. Levin, R. W. Young, and R. L. Stohler, “Estimation of Median Human Lethal Radiation Dose Computed from Data on Occupants of Reinforced Concrete Structures in Nagasaki, Japan,” *Health Physics* 63 (1992): 522–31.

<sup>6</sup> A.K. Guskova et al., “Acute Effects of Radiation Exposure Following the Chernobyl Accident,” in *Treatment of Radiation Injuries*, ed. D. Browne et al. (New York: Plenum Press, 1990).

<sup>7</sup> The DRF is calculated by dividing the LD<sub>50</sub> with treatment by the LD<sub>50</sub> without treatment.

<sup>8</sup> G.H. Anno et al., “Dose Response Relationships for Acute Ionizing-radiation Lethality,” *Health Physics* 84 (2003): 565–75.

platelet replacement.<sup>9</sup> The DRF at the LD<sub>50/30</sub> for supportive care was 1.3 for cobalt-60 (Co-60) gamma-IR. In the same study, the DRF for supportive care using mixed fission-neutron-gamma IR was 1.21.<sup>10</sup>

A third evaluation of the effects of supportive care was performed by comparing a current NHP IR study with supportive care to two historical studies without supportive care. Most NHP studies today require some supportive care measures based on the regulations of the Institutional Animal Care and Use Committee (IACUC) of the institute performing the animal studies, so most countermeasures tested in the United States on NHPs will require some supportive care by default. The original NHP ARS studies without supportive care used Co-60<sup>11</sup> and two mega electron volt (MeV) x-rays.<sup>12</sup> Farese et al. used these in comparing the LD<sub>50/60</sub> in NHP using a linear accelerator (LINAC) and estimated a DRF of 1.13.<sup>13</sup> Furthermore, in an NHP study comparing LD<sub>50/30</sub> with 250 peak kilovoltage (kVp) x-irradiation in 418 animals without supportive care (from the literature) and 72 animals used in the Farese and MacVittie labs,<sup>14</sup> an approximate DRF for supportive care would be 1.45.<sup>15</sup>

Considering the quality and quantity of these data from NHPs and canines and incorporating the various studies in Anno et al., the IDA research team estimates the DRF for supportive care to be 1.5 in humans at the LD<sub>50/60</sub>.<sup>16</sup> A DRF of 1.5 changes the LD<sub>50/60</sub> for humans from 450 cGy to 675 cGy. See Appendix A for an explanation of how a DRF of 1.5 could change fatalities following a nuclear incident.

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<sup>9</sup> Triggers to treat are described within the paper, MacVittie et al., “The Relative Biological Effectiveness of Mixed Fission-Neutron-Gamma Radiation on the Hematopoietic Syndrome in the Canine: Effect of Therapy on Survival,” *Radiat Res* 128 (1991): S29–36.

<sup>10</sup> Ibid.

<sup>11</sup> J. R. Eltringham, “Recovery of the Rhesus Monkey from an Acute Radiation Exposure as Evaluated by the Split-Dose Technique: Preliminary Results,” (no journal, no year); communicated through Thomas Elliott and William Blakely (AFRR); cited as an abstract in *Radiat Res* 31 (1967): 533.

<sup>12</sup> G. V. Dalrymple, I. R. Lindsay, and J. J. Ghidoni, “The Effect of 2-Mev Whole-body x-Irradiation on Primates,” *Radiat Res* 25 (1965): 377–400.

<sup>13</sup> A.M. Farese et al., “A Nonhuman Primate Model of the Hematopoietic Acute Radiation Syndrome Plus Medical Management,” *Health Physics* 103 (2012): 367–82.

<sup>14</sup> Farese et al., “Pegfilgrastim Administered in an Abbreviated Schedule, Significantly Improved Neutrophil Recovery after High-Dose Radiation-Induced Myelosuppression in Rhesus Macaques,” 403–13.

<sup>15</sup> Calculated from Farese et al., “Pegfilgrastim Administered in an Abbreviated Schedule, Significantly Improved Neutrophil Recovery after High-Dose Radiation-Induced Myelosuppression in Rhesus Macaques,” by taking the LD<sub>50/30</sub> with supportive care (7.18) and dividing it by the LD<sub>50/30</sub> without supportive care (4.93).

<sup>16</sup> Anno et al., “Dose Response Relationships for Acute Ionizing-Radiation Lethality,” 565–75.

While managing the symptoms of ARS with supportive care can enhance the prognosis of the patient, it does not generally treat the underlying cellular and tissue damage from IR. Although very high doses of IR lead to a rapid demise via neurovascular failure, the cause of death in HP and GI subsyndromes is usually sepsis. Prevention or rapid treatment of sepsis in the HP subsyndrome allows extra time for the patient to recover from the IR-induced cell damage to the HP system, if recovery is possible. However, treatments that would prevent cell and tissue damage or aid in the repair of the damage would speed recovery and could change the symptom progression.

## Appendix C

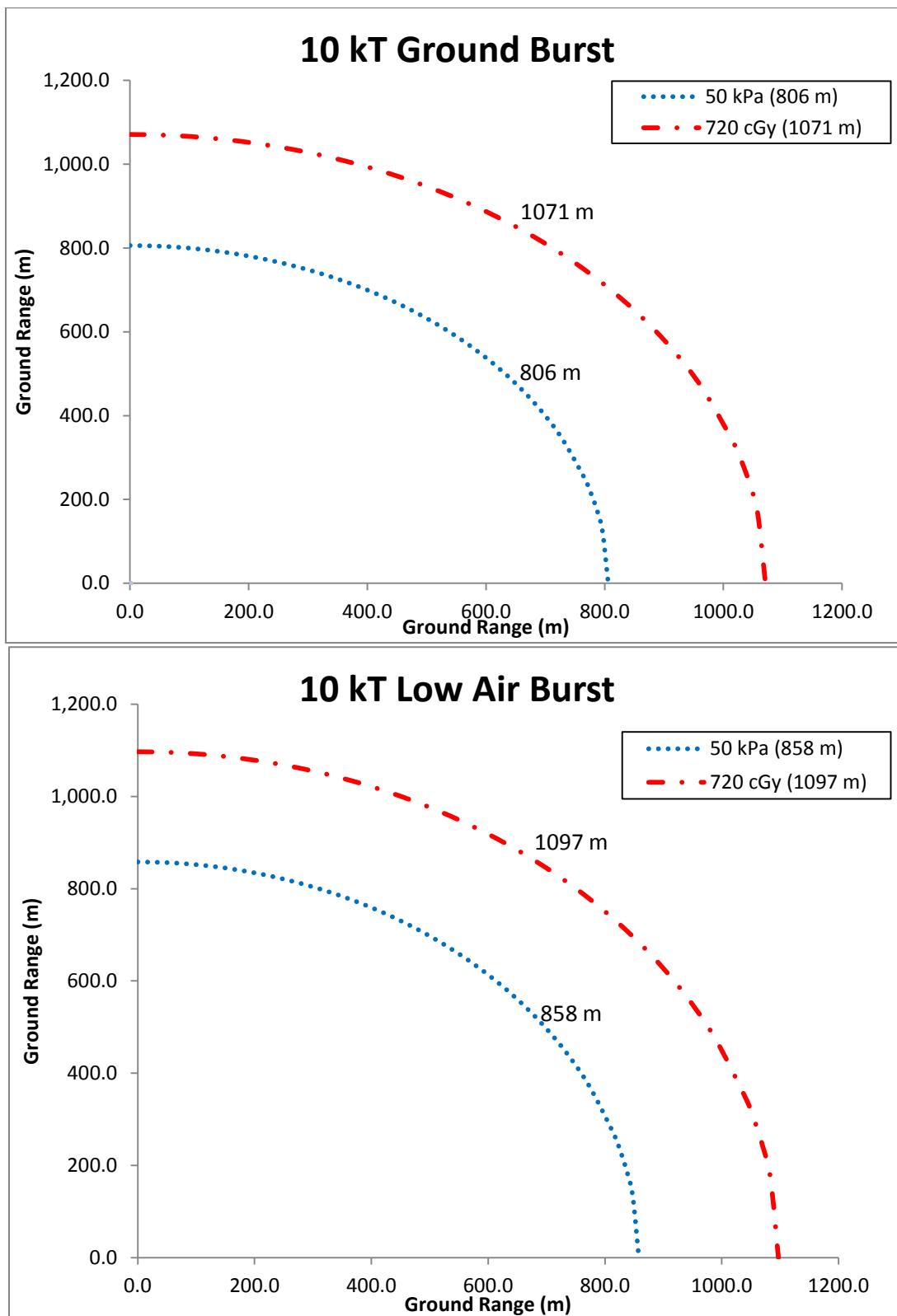
### **Using *AMedP-8(C)* Casualty Estimate Methodology to Model Changes in Fatalities from the Implementation of Radiation Medical Countermeasures**

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The estimation of fatalities described here follows *AMedP-8(C)* methodology<sup>1</sup> in assuming that contours for overpressure in kilopascals (kPa), thermal fluence in kilojoules per meter squared (kJ/m<sup>2</sup>), and radiation exposure in centiGray (cGy) (referred to hereafter as *nuclear effects*) subsequent to a nuclear weapon detonation are observed in concentric circles situated around the location at which the nuclear weapon was detonated. For a given measure of nuclear effect, the radius of the circular contour associated with that measure changes depending on the yield and burst height of the nuclear weapon. Figure C-1 depicts an example of differing radii for 50 kPa and 720 cGy contours corresponding to 10 kiloton (kT) ground and low air bursts.

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<sup>1</sup> North Atlantic Treaty Organization (NATO). *AMedP-8(C): NATO Planning Guide for the Estimation of CBRN Casualties*, STANAG 2553, March 2011.



**Figure C-1. Extent to Which a Measure of Nuclear Effect is Observed Depends on the Conditions of the Nuclear Detonation**

*AMedP-8(C)* provides further guidance for associating measures of nuclear effects with observed human outcomes. Using this guidance and defining fatality as any person who was exposed to a level of a nuclear effect sufficient to be classified as Died of Wounds (DoW), any person who has been exposed to 290 kPa of overpressure or 450 cGy of radiation or received second-degree burns on 30% of their body's skin surface area (% BSA [percent of body surface area]) due to thermal fluence<sup>2</sup> is considered a fatality. As a consequence of earlier assumptions, the areas in which people will become fatalities as a result of nuclear effects will be concentric circles around the detonation point. However, the radius associated with 290 kPa is less than the radius associated with 30% BSA for all combinations of yield and burst height considered in this study, implying that the cohort of overpressure fatalities will always be a subset of the cohort of thermal fatalities. Thus, overpressure fatalities need not be considered.

The effect on radiation fatalities due to use of the radiation treatment filgrastim and supportive care was explored by considering the change in maximal distance from the detonation point at which radiation fatalities are observed. Treatment with filgrastim and supportive care is estimated to have a dose reduction factor (DRF) of 1.88; applying this to the radiation exposure threshold of 450 cGy yields a new, higher threshold of 846 cGy, which will correspond to a reduced radius of radiation fatalities for any combination of yield and burst height. Table C-1 contains, for each yield and burst height, the maximal distance from the detonation point at which fatalities will be observed due to thermal burn, untreated radiation, and radiation treated with filgrastim and supportive care.

**Table C-1. Maximal Distance (in Meters) from the Point of Detonation at which Fatalities Occur Due to Nuclear Effects**

	Ground Burst			Low Air Burst		
	1 kT	10 kT	50 kT	1 kT	10 kT	50 kT
<b>Burn DoW (30% BSA)</b>	223	718	1595	336	1052	2270
<b>Rad DoW w/ DRF 1.88 (846 cGy)</b>	691	1045	1392	711	1071	1395
<b>Rad DoW w/ DRF 1 (450 cGy)</b>	774	1147	1512	795	1437	1515
<b>Predominating Effect</b>	Rad	Rad	Burn	Rad	Rad	Burn

<sup>2</sup> The conversion to % BSA from thermal fluence assumes 12% of the person's body is unprotected while the remainder is covered by clothing with a transmissivity comparable to a military Battle Dress Uniform (BDU) with an undershirt and airflow.

For the purpose of fatality estimation a Visual Basic for Applications (VBA) macro was created that accepts as input a pair of kilometer ranges of minimum and maximum ( $k_{min}$ ,  $k_{max}$ ) in addition to a list of grid points in kilometers and the population count at each grid point. On execution, the macro iterates over a user-specified set of grid points and calculates the total population within the annulus defined by ( $k_{min}$ ,  $k_{max}$ ) about each of the specified points. The output from this process is a frequency table with each unique calculated total population value and the number of grid points that contained that total population within their ( $k_{min}$ ,  $k_{max}$ ) annuli. Using this macro, a range of radiation-only fatality estimates were derived for each yield and burst height by inputting for ( $k_{min}$ ,  $k_{max}$ ) each of

- ([thermal burn radius], [untreated radiation radius])
- ([thermal burn radius], [treated radiation radius])

Population data for Washington, DC and a prototypical Heavy Brigade Combat Team in the offensive (HBCT-O) were used as population inputs and were obtained from LandScan data<sup>3</sup> and *Chemical and Biological Defense Planning Scenarios*, respectively.<sup>4</sup> For each yield and burst height the reported radiation fatality values correspond to the 90th percentile fatality values. Reported values for fatalities due to all nuclear effects were calculated by adding the total population in the interval (0, [thermal burn radius]) about the grid point corresponding to the 90th percentile of untreated radiation fatalities; when multiple grid points met the criteria the median of the total populations in each (0, [thermal burn radius]) annulus was chosen.

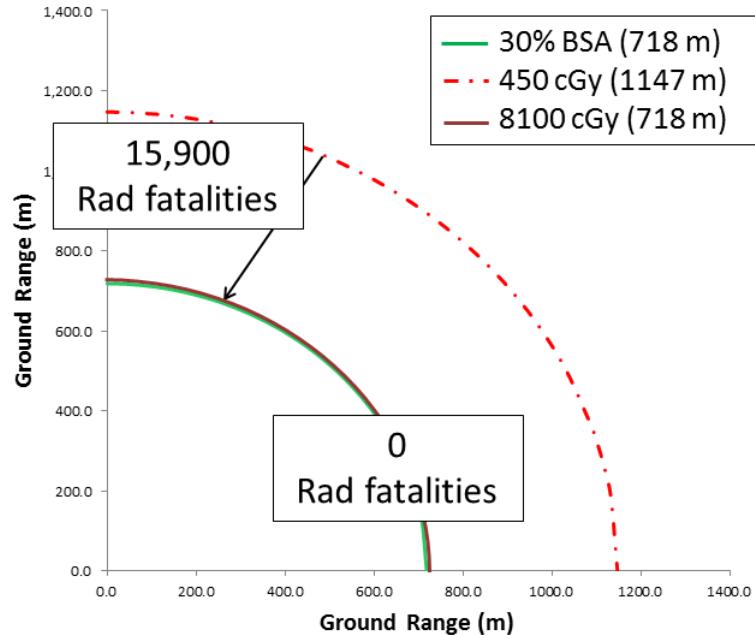
The optimal DRF for each yield and burst height was calculated by taking the ratio of observed radiation exposure (in cGy) at the thermal burn radius and 450 cGy, the level of radiation exposure at which untreated individuals will become fatalities. Mathematically, the result of this calculation yields a radius of radiation fatalities is compressed to equal the radius of burn fatalities (see Figure C-2); practically, this means that no fatalities due to radiation alone will be observed since all individuals who would be radiation fatalities will succumb to thermal burn effects. This justifies the optimality of this calculated DRF—further increasing the DRF would not further reduce the number of fatalities because everyone who would benefit is a fatality due to burn effects anyway.

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<sup>3</sup> This product was made utilizing the LandScan (2012) High Resolution Global Population Data Set copyrighted by UT-Battelle, LLC, operator of Oak Ridge National Laboratory under Contract No. DE-AC05-00OR22725 with the United States Department of Energy. The United States Government has certain rights in this Data Set. Neither UT-Battelle, LLC nor the United States Department of Energy, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of the data set.

<sup>4</sup> J.H. Grotte et al., “Chemical and Biological Defense Planning Scenarios,” IDA Paper P-4629 (Alexandria, VA: IDA, 2012), v, SECRET. (Only Unclassified information used.)

The calculation for observed radiation exposure at the thermal burn radius originates from the same source from which the values in Table C-1 were derived.



**Figure C-2. 10 kT Ground Burst: Limiting DRF for Radiation Fatalities**



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## Appendix E

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## Appendix F

### Abbreviations

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AFRRI	Armed Forces Radiobiology Research Institute
<i>AMedP-6(B)</i>	<i>Allied Medical Publication-6(B)</i>
<i>AMedP-8(C)</i>	<i>Allied Medical Publication-8(C)</i>
AMQP	Animal Model Qualification Program
ANC	Absolute Neutrophil Count
ARS	Acute Radiation Syndrome
% BSA	percent of body surface area
BARDA	Biomedical Advanced Research & Development Authority
BDP	beclamethasone dipropionate
BDU	battle dress uniform
BEIR	Biological Effects of Ionizing Radiation
CBER	Center for Biologics Evaluation and Research
CBRN	Chemical, Biological, Radiological, and Nuclear
CCFA	Crohn's and Colitis Foundation of America
CDER	Center for Drug Evaluation and Research
CDC	Centers for Disease Control and Prevention
cGy	centiGray
Co-60	cobalt-60
COU	context of use
Cs-137	cesium-137
CSF	colony-stimulating factor
CV	cerebrovascular
DHHS	U.S. Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	U.S. Department of Defense
DOW	Died of Wounds
DRF	dose reduction factor
<i>E. coli</i>	<i>Escherichia coli</i>
EPO	erythropoietin
FDA	U.S. Food and Drug Administration

FIA	free in air
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte monocyte colony-stimulating factor
GI	gastrointestinal
GLP	good laboratory practice
GMP	good manufactory practice
GvHD	graft-versus-host disease
HBCT-O	heavy brigade combat team in the offensive
HDAC	histone deacetylase
HP	Hematopoietic
HTS	high throughput screen
IACUC	Institutional Animal Care and Use Committee
IBD	inflammatory bowel disease
IDA	Institute for Defense Analyses
IL-3	interleukin-3
IL-11	interleukin-11
IL-12	interleukin-12
IND	investigational new drug
IR	ionizing radiation
Ir-192	iridium-192
ITP	idiopathic thrombocytopenia purpura
IV	intravenous
kg	kilogram
KGF	keratinocyte growth factor
$\text{kJ/m}^2$	kilojoules per meter squared
$k_{min}$	minimum kilometers
$k_{max}$	maximum kilometers
kPa	kilopascals
kVp	peak kilovoltage
L	liter
LD <sub>50</sub>	median lethal dose
LD <sub>50/30</sub>	median lethal dose at 30 days
LD <sub>50/60</sub>	median lethal dose at 60 days
LGF	leukocyte growth factor
LINAC	linear accelerator
LNT	linear non threshold

METREPOL	Medical Treatment Protocols for Radiation Accident Victims
MeV	Mega electron Volt
$\mu$ g	microgram
MODS	multi-organ dysfunction syndrome
MOF	multi-organ failure
NATO	North Atlantic Treaty Organization
NCATS	National Center for Advancing Translational Sciences
NCI	National Cancer Institute
NF $\kappa$ B	nuclear factor-kappa B
ng	nanogram
NHP	non-human primate
NIAID	National Institute for Allergies and Infectious Diseases
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
NIMS	National Incident Management System
OTSG	Office of the Surgeon General (U.S. Army)
PBPC	peripheral blood progenitor cells
PD	pharmacodynamics
PEG	polyethylene glycol
peg-rHuMGDF	pegylated recombinant human megakaryocyte growth and development factor
PK	pharmacokinetics
R&D	research and development
RBC	red blood cell
REMM	Radiation Emergency Medical Management
rHuIL-12	recombinant human interleukin-12
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
sBLA	supplemental Biological Licensure Application
SEER	Surveillance, Epidemiology, and End Results
SNS	Strategic National Stockpile
TBI	total body irradiation
TLR2	toll-like receptor 2
TLR5	toll-like receptor 5
TLR6	toll-like receptor 6
TPO	thrombopoietin

UNSCEAR	United Nations Scientific Committee on the Effects of Atomic Radiation
U.S.	United States
USD	United States Dollars
USG	U.S. Government
VBA	Visual Basic for Applications

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<b>14. ABSTRACT</b>  The U.S. Army Office of the Surgeon General (OTSG) asked the Institute of Defense Analyses (IDA) to review radiation medical countermeasures (task CA-6-3079), such as radioprotectant drugs and radiation injury treatments, and their potential to mitigate the effects of a radiation or nuclear incident on the health and survival of those exposed to ionizing radiation (IR). Although no radioprotectants or radiation injury treatments are currently approved by the U.S. Food and Drug Administration (FDA) for the treatment of acute radiation syndrome (ARS), this paper describes the treatment of ARS with supportive care and filgrastim and includes an illustrative example of how these treatments could affect the number of casualties from a nuclear event. In addition to describing some example potential therapies that are still being tested for safety and efficacy, this paper outlines strategies beyond the traditional research and development (R&D) pharmaceutical model that the Department of Defense could implement for acquiring future treatments for ARS. If pursued, these alternate R&D paradigms could provide significant cost and time savings to the DOD as well as reduce the risk associated with pursuing the approval of a radiation therapy or radioprotectant.					
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